APPENDIX A

(Clean Version)

A Genomic Approach to Identification of Novel Broad-spectrum Antimicrobial Peptides From Bony Fish

The present application is a 371 of PCT/CA2003/001323 and claims priority under 35 U.S.C. §119 to U.S. Application Serial No. 60/404,922 filed August 22, 2002.

BACKGROUND OF THE INVENTION

Antimicrobial peptides have been isolated from a wide variety of plants and animals, and play an important role in defense against microbial invasion. They fall into three main classes based on secondary structure and amino acid sequence similarities: α-helical structures, highly disulphide-bonded (cysteine-rich) β-sheets and those with a high percentage of single amino acids such as proline or arginine. Most molecules are amphiphilic and contain both cationic and hydrophobic surfaces, enabling them to insert into biological membranes. Although one of the modes of action of antimicrobial peptides has been described as lysis of pathogens, they may also exert their effects by binding to intracellular targets. They have also been reported to exert a number of effects such as mediating inflammation and modulating the immune response.

A small number of natural antimicrobial peptides have been isolated from teleosts including the pleurocidin, from the skin of winter flounder (Cole, Weis et al. 1997), pardaxin from Red Sea Moses sole (Oren and Shai 1996), misgurnin from loach (Park, Lee et al. 1997), HFA-1 from hagfish (Hwang, Seo et al. 1999), piscidins from hybrid striped bass eosinophilic granule cells (Silphaduang and Noga 2001), moronecidins from hybrid striped bass (Lauth, Shike et al. 2002), parasin, a cleavage product of histone 2A from catfish (Park, Park et al. 1998) and some uncharacterized mucous secretions from carp (LeMaitre, Orange et al. 1996) and trout (Smith, Fernandes et al. 2000). In addition, a cationic steroidal antibiotic, squalamine, has been isolated from the shark, *Squalus acanthias* (Moore, Wehrli et al. 1993).

Cysteine-rich antimicrobial peptides of the defensin family have been detected in the fat body of insects and the hemolymph of molluscs and crustaceans. They have also been isolated from various epithelia of mammals as well as circulating cells such as neutrophils and macrophages. Recently, small cysteine-rich peptides exhibiting antimicrobial activity against various fungi, Gram positive and Gram negative bacteria have been isolated from blood ultrafiltrate (Krause, Neitz et al. 2000), the human urinary tract (Park, Valore et al. 2001), and the gill of bacterially challenged hybrid striped bass (Shike et al. 2002). These peptides, referred to as hepcidin or LEAP-1 (liver-expressed antimicrobial peptide), have been proposed to be the vertebrate counterpart of insect peptides induced in the fat body in response to infection (Park, Valore et al. 2001).

Antimicrobial peptides have a variety of potential uses. (see for example US 6,288,212 of Hancock)

The conventional approach to identifying antimicrobial peptides involves biochemical purification from tissues or secretions. Fractions are tested for antimicrobial activity, and the purified peptides that exhibit activity are then sequenced. This approach is costly, time consuming, and not well suited to the identification of low abundance or difficult-to-purify antimicrobial peptides.

Thus, it is an object of the invention to provide a method for identifying potential antimicrobial peptides.

SUMMARY OF THE INVENTION

In one aspect of the invention there is provided a method of identifying candidate nucleic acid sequences encoding antimicrobial peptides, said method comprising:

(a) identifying an initial peptide of interest;

(b) identifying genomic DNA encoding the initial peptide;

(c) identifying a flanking sequence on each side of the initial peptide;

(d) obtaining primers complementary to the flanking sequences; and,

(e) screening a wide range of nucleic acid sequences to identify candidate sequences capable of being amplified using the primers from step d).

According to one aspect of the invention the nucleotide and deduced amino acid sequences of hepcidin-like peptides are provided.

According to another aspect of the invention, the nucleotide and deduced amino acid sequences of pleurocidin – like peptides are provided.

According to another aspect of the invention primers suitable for use in the identification, isolation and/or amplification of nucleic acid sequences encoding novel microbial peptides are provided.

According to another aspect of the invention there is provided a method for the identification of families of nucleic acid sequences encoding antimicrobial peptides.

BRIEF DESCRIPTION OF THE DRAWINGS

*Figure.*1 is a textual and graphical depiction of pleurocidin WF2 cDNAfrom winter flounder (A), a graphical depiction of a predicted hydrophobicity plot of peptide WF2 (B), and a diagrammatic depiction of a predicted helical structure of WF2 (C).

Figure 2 is a pictorial depiction of results of amplification of certain hepcidin-like cDNAs.

Figure 3 is a depiction of certain aligned pleurocidin –like peptide sequences.

Figure 4 is a pictorial depiction of the results of PCR amplification of certain pleurocidin-like genomic sequences.

Figure 5 is a depiction of an extended genomic sequence of WF4.

Figure 6 is a depiction of an alignment of certain pleurocidin-like polypeptide sequences.

Figure 7 is a pictorial depiction of the results of expression of certain pleurocidin-like genes in different winter flounder tissues.

Figure 8 is a pictorial depiction of the results of RTPCR of expression of certain pleurocidins during winter flounder development.

Figure 9 is a pictorial depiction of the results of a study of the expression of certain pleurocidin-like genes during winter flounder development.

Figure 10 is a pictorial depiction of the results of a Southern analysis of certain pleurocidin genes of winter flounder.

Figure 11 is a schematic depiction of the genomic organization of certain pleurocidin genes from winter flounder.

Figure 12 is a schematic depiction of certain transcription factor binding sites located upstream from pleurocidin genes from winter flounder.

Figure 13 is a graphical depiction of results showing the impact of peptide NRC-15 on bacterial survival.

Figure 14 is a graphical depiction of results showing the impact of peptide NRC-13 on bacterial survival.

Figure 15 is a graphical depiction of results showing the impact of peptide NRC-12 on yeast survival.

Figure 16 is a depiction of nucleotide sequences of an unspliced (A) and partially spliced (B) cDNA encoding a type I hepcidin and a schematic depiction of intron/exon structure of a hepcidin gene in human, mouse and salmon (C).

Figure 17 is a depiction of certain hepcidin sequences from different species shown in alignment.

Figure 18 is a depiction of certain aligned 3' untranslated regions of hepcidin genes from winter flounder (A) and Atlantic salmon (B).

Figure 19 is a pictorial depiction of the results of Southern hybridization analysis of certain hepcidins from different fish species.

Figure 20 is a pictorial depiction of the results of an assay of the expression of certain hepcidin and actin genes in various tissues of winter flounder.

Figure 21 is a pictorial depiction of the results of an assay of the expression of certain Type I (A) and Type 2 (B) hepcidin and actin genes in various tissues of control and infected salmon.

Figure 22 is a pictorial depiction of the results of an assay of expression of certain Type I (A), Type II (B) and Type III (C) hepcidin and actin genes in developing winter flounder larvae.

Figure 23 is a schematic depiction of steps taken in an embodiment of the method for identifying pleurocidins.

Figure 24 is a schematic depiction of steps taken in an embodiment of the method for identifying hepcidins.

Figure 25 is a graphical depiction of experimental results using antimicrobial peptide NRC-13 in the presence of I50 mM NaCe.

DETAILED DESCRIPTION OF THE INVENTION

The method of the invention builds on the surprising discovery that the flanking sequences around antimicrobial peptides, including without limitation pleurocidins and hepcidins, are conserved. The method of the invention provides a means of identifying nucleotide sequences encoding pleurocidins and hepcidins, and identifying the encoded polypeptide sequences.

In one embodiment, the method provides, generally, a way of identifying members of a family of antimicrobial peptides once a single family member has been identified. The initial family member may be an initial peptide of interest. Initial peptides of interest can be identified based on either known or reported antimicrobial activity or based on sequence similarity to other known antimicrobial peptides. Once an initial peptide has been identified, the genomic DNA encoding it is identified and its flanking sequences are determined.

As used herein, the term "flanking sequences" refers to nucleic acid sequences appearing at or near one or both ends of a target nucleic acid sequence encoding an antimicrobial peptide.

As used herein a nucleic acid sequence is "at or near" the end of a target sequence if a portion of the sequence is within 50 nucleic acids of the end of the gene (whether within the coding region or outside it).

When an initial peptide of interest is identified based on sequence similarity to another peptide with known antimicrobial activity, the initial peptide preferably has an amphipathic structure and a net charge. In some instances the charge will preferably be a net positive charge of at least 2. In some instances, the peptide is at least 75 %, 85% or 95 % identical in sequence to the peptide having known antimicrobial activity. In some instances the sequence similarity identified may relate to similarity between nucleic acid sequences encoding the known peptide and encoding the peptide of interest. In such instances, the predicted peptide for the peptide of interest will be considered with respect to predicted charge and amphipathic structure.

For example, the prepro-sequences of pleurocidins and hepcidins tend to be conserved. Thus, by employing nucleic acid primers specific for such sequences, one can identify potential pleurocidin- and hepcidin- encoding sequences. Alternatively or additionally, known gene sequences of other classes of antimicrobial peptides can be examined to identify regions which appear to encode conserved prepro-sequences and a similar strategy used to identify other members of this family of peptides. The corresponding antimicrobial peptide encoded by such sequences can be predicted using the general features found in most pleurocidins and hepcidins, such as, for example, a net positive charge of at least 2 and an amphipathic structure.

As used herein with respect to pre-, pro- and prepro sequences of antimicrobial peptides, "pre" and "pro" have the following meaning: "Pre" refers to the signal peptide portion (or a functional portion thereof) of the peptide. "Pro" refers to the propiece. In pleurocidins the propiece is the anionic region at the carboxy terminus. In hepcidins the propiece is the region upstream of the mature peptide. In the non-limiting examples disclosed herein pleurocidin primers were designed based on the

pre and pro regions, and hepcidin primers were designed based on the pre region and the 3' untranslated region (UTR).

PCR can be used to amplify nucleic acid sequences encoding potential pleurocidins or hepcidins. This can be conveniently accomplished by using a pair of PCR primers, one of which recognises a nucleic acid sequence complementary to a polynucleotide sequence encoding an amino-terminal prepro-sequence conserved in the peptide type of interest, and the other complementary to a 3' conserved region in the nucleotide encoding the peptide-type of interest. It will be appreciated that other prepro-sequences may exist and are specifically contemplated. For example, redundancy in the genetic code allows for multiple nucleic acid sequences encoding a particular amino acid sequence. As discussed with respect to 5'prepro-sequences, other 3' conserved sequences may exist and are specifically contemplated. When designing primers it is useful to have reference to known codon usage information for the species in which sequence amplification is sought.

In an embodiment of the invention there is provided the use of signal sequence I or a nucleic acid sequence encoding same in identifying or amplifying potential pleurocidins.

Signal Sequence I (SEQ ID NO: 305)

$MKFTATFL\ (X)_n\ (L)_o\ (F)_p\ I\ (F)_q\ (X)_y\ VLM\ (X)_z\ (V)_r\ (E)_s\ (D)_t\ (P)_u\ (L)_v\ G\ E\ (C)_w\ (G)_x$

Wherein:

n is 1 to 3	u is 0 or 1
o is 0 to 2	v is 0 or 1
p is 0 or 1	w is 0 or 1
r is 0 or 1	
s is 0 or 1	x is 0 or 1
t is 0 or 1	y is 0 or 1
	z is 0 or 1

with the restriction that:

$x + o + p = 3$,	$s + t = 1$,
$u + v = 1$,	$w + x = 1$, and
$q + = 1$.	

In an embodiment of the invention there is provided the use of one or both sequence PL1 or PL2 in identifying or amplifying potential pleurocidins.

PL1 GCCCACTTTGTATTCGCAAG (SEQ ID NO: 5)

PL2 CTGAAGGCTCCTTCAAGGCG (SEQ ID NO: 6)

In an embodiment of the invention there is provided the use of an acidic sequence I or a nucleic acid sequence encoding same in identifying or amplifying potential pleurocidins.

Acidic Sequence I (SEQ ID NO: 306)

$(Y)_a (X)_b (X)_c (E)_d (X)_e (Q)_f (E)_g$ L (N/D) KR (A/S) V D (D/E)

wherein:

a is 0 or 1
b is 0 or 1
c is 1 or 2
d is 0 or 1
e is 1 to 3
f is 0 or 1
g is 0 or 1

with the restriction that

$a + b = 1$,

$c + d = 2$, and

$e + f + g = 3$.

As used in the sequences herein "X" refers to any amino acid. Nucleic acid sequences encoding signal sequence I and acidic sequence I are specifically contemplated, as are nucleic acid sequences complementary to such nucleic acid sequences.

In an embodiment of the invention there is provided the use of signal peptide II, III, IV, V or a nucleic acid encoding same, in the identification or amplification of hepcidins.

Signal Peptide II

MKXXXXAXXVXXVL (SEQ ID NO: 307)

Signal Peptide III

MKTFSVAV (SEQ ID NO: 308)

Signal Peptide IV

MKTFSVAVTVAVVLXFICIQQSSA (SEQ ID NO: 309)

Signal Peptide V

MKTFSVAVAV (T/V) (L/V) VLA $(F)_n$(V/C) (C/M) (I/F) (Q/I) X $(X)_m$ S (S/T) AV P F XXV (SEQ ID NO: 310),

Wherein n is 0 or 1 and m is 0 or 1.

In an embodiment of the invention there is provided the use of prosequence I, Prosequence II or a nucleotide sequence encoding same or complementary to one encoding same in the identification or amplification of hepcidins.

Prosequence I

PEVQXLEEAXSXDNAAAEHQE (SEQ ID NO: 311)

Prosequence II

PFXXVX$(X)_n$ (L/T) EEV (E/G) (G/S) XD (T/S) PV (A/G) XHQ (SEQ ID NO: 312),

Wherein n is 0 or 1,

In an embodiment of the invention there is provided the use of HcPA3b3' and/or HcSal3' in the identification or amplification of hepcidins.

HcPa3b 3' 3'ACAACCTCGTCCTTAGG5' (SEQ ID NO: 313)

HcSal 3' 3'ACGCCCGTCCAGGAAT5' (SEQ ID NO: 314)

Non-limiting Examples Of Uses

Antimicrobial peptides are useful in the treatment and/or prevention of infection in a variety of subjects, including fish, reptiles, birds, mammals, amphibians and insects.

Antimicrobial peptides are also useful for reducing bacterial growth and/or accumulation on surfaces. This is of particular benefit in the food industry where antimicrobial peptides can be used for coating surfaces used in the processing, preparation, and/or packaging of food.

Antimicrobial peptides disclosed herein can be administered in a variety of ways. In some instances, oral administration will be desirable. Some types of oral administration will be improved by encapsulation of the peptides so as to allow their preferential release at a particular stage in digestion. In some instances it will be desirable to include pre and/or pro sequences in the administered peptide (for example to improve stability or modulate activity). The pre and/or pro sequences can be cleaved off by endogenous proteases at the appropriate stage. Peptides may be administered by inhalation where the subject breathes air or by addition to water for gilled subjects. Administration by injection will in some cases be desirable. Peptides may be injected into any number of sites. In some cases intravenous injection will be desired. In some instances injection directly into or adjacent to the site of infection or potential infection will be desired. In some instances topical administration will be desired. Where the presence of the antimicrobial peptide is desired at a remote and specific site, or where the peptide will be desired for a prolonged period of time, gene therapy may be used to provide expression of one or more antibacterial peptides in the tissue(s) of concern.

Where the subject is a cultured or domesticated creature such as a fish, bird or non-human mammal, production of a transgenic variety which expresses one or more antibacterial peptides may be desired. Methods for producing transgenic animals are well known. (See for example Mar.Biotechnol.4: 338,2002).

A variety of antimicrobial peptides are contemplated and fall within the scope of the invention. By way of non-limiting example, peptides comprising the following

amino acid sequences or a sequence at least 80% or 90% homologous thereto, and nucleic acid sequences encoding them are specifically contemplated:

i) GW(G/K)XXFXK (SEQ ID NO: 315)

ii) GXXXXXXXHXGXXIH (SEQ ID NO: 316)

iii) FKCKFCCGCCXXGVCGXCC (SEQ ID NO: 317)

iv) CXXCCNCC (K/H) XKGCGFCCKF (SEQ ID NO: 318)

v) FKCKFCCGCRCGXXCGLCCKF (SEQ ID NO: 319)

vi) XXXCXXCCNXXGCGXCCKX (SEQ ID NO: 320)

Other specific, non-limiting examples of antimicrobial sequences of interest can be found in Tables 4 and 11.

Antimicrobial peptides of the invention may be modified. Such modifications may in some instances improve the peptides' stability or activity. Examples of modifications specifically contemplated include:

- conservative amino acid substitutions (acidic with acidic, basic with basic, neutral with neutral, polar with polar, hydrophobic with hydrophobic, etc.)

- addition of positively charged amino acids (lysine, arginine, histidine) at either or both ends

- replacement of amino acids with others unlikely to result in structural changes including D-amino acids and peptidomimetics

- deletion of one or more amino acids

- modifications at C-terminal or N-terminal ends, including methyl esters and amidates

- cyclised versions of the peptides (which may result in increased stability without adversely affecting activity)

Examples – Methods

Fish Rearing

Winter flounder larvae were reared as described (Douglas, Gawlicka et al. 1999), the disclosure of which is incorporated herein by reference. Saint John River stock Atlantic salmon (*Salmo salar* L.) were maintained in single-pass, heated, dechlorinated fresh water at 12°C in the Dalhousie University Aquatron facility in Halifax, Nova Scotia. All fish were euthanised with an overdose of tricaine methanesulfonate (MS 222, 0.1 g L^{-1}, Argent Chemical Laboratories, Inc., Redmond,

WA, USA) prior to sampling. All animal procedures were approved by the Dalhousie University Committee for Laboratory Animals and the National Research Council - Halifax Local Animal Care Committee.

Bacterial Challenge

Aeromonas salmonicida subsp *salmonicida* strain A449 (Trust et al. 1983) was cultured to mid-logarithmic growth in Tryptic Soy Broth (TSB) at 17°C. The absorbance at 600nm of the bacterial suspension was determined and the bacteria were resuspended to approximately 5 x 10^7 cfu mL^{-1} in sterile Hanks Balanced Salt Solution (HBSS). Three salmon (200g each) were anaesthetised with 50 mg L^{-1} TMS, injected intraperitoneally with 2.5 x 10^6 cfu bacteria in 50 µL HBSS and allowed to recover in fresh water. Uninjected fish from the same cohort were maintained in separate tanks as controls. Three days post-injection, control and infected salmon were euthanised as described above and samples of tissues removed. Blood was drawn from the caudal vein into a heparinised container. To confirm that the fish were positive for *A. salmonicida*, the posterior kidney of both infected and control fish were swabbed and used to inoculate tryptic soy agar (TSA) that was incubated at room temperature overnight. Atlantic halibut tissue samples were obtained from a bacterial challenge study performed at Bedford Institute of Oceanography, Dartmouth, Nova Scotia.

Sampling

Tissues (oesophagus, stomach, pyloric caecae, liver, spleen, intestine, anterior kidney, posterior kidney, gill, skin, ovary, rectum, heart, muscle and brain) were removed into RNALater (Ambion, Austin, TX, USA) and kept at -80° C until used. Samples of winter flounder larvae at different stages and juveniles were rinsed in RNALater (Ambion, Austin, TX, USA), transferred into 1.5 ml Eppendorf tubes containing 0.5-1.25 ml RNALater, and kept at -80° C until used.

Pleurocidins

The general approach followed is shown in Figure 24

Isolation of pleurocidin cDNA

A cDNA library constructed from winter flounder skin (Gong et al 1996) was screened using degenerate oligonucleotides (PleuroA, PleuroB; Table 1). The library was plated at 80,000 phage/plate and duplicate lifts to HyBond filters were made of each of eight plates. A mixture of radioactively end-labelled PleuroA and PleuroB probes was hybridised with the filters at 50° C using standard procedures, and the filters were washed in 1X SSC/0.1% SDS at 50° C for 45 min. Plaques that showed matching hybridization signals on both duplicate filters were picked and the library rescreened until 100% purity of the recombinant plaques was obtained. Two recombinants were completely sequenced using an ABI373 stretch automated sequencer and the AmpliTaqFS Dye Terminator Cycle Sequencing Ready Reaction kit (Perkin-Elmer, Foster City, CA, USA). Sequence data were analyzed using Sequencher (Gene Codes, Inc., Ann Arbor, MI, USA) and DNA Strider. The amino-terminal signal sequence was predicted using SignalP (http://www.cbs.dtu.dk/services/SignalP). The Helical Wheel routine of the GCG package (http://www.gcg.com) was used to model the helical structure of the predicted antimicrobial peptide sequences.

Genomic PCR

Genomic sequences were amplified using two sets of primers specific to the winter flounder pleurocidin cDNA (PL1/PL2 and PL5'/PL3'; Table 1; Fig. 1). The amplification conditions were: 1 min at 94° C; 35 cycles of 30 s at 94° C; 30 s at 52° C, 90 s at 72° C; and 2 min at 72° C, and products were resolved on a 1% agarose gel. Bands were excised from the gel, extracted using Gene-Clean (Bio101, La Jolla, CA, USA) and cloned into the Topo TA2.1 vector (Invitrogen, Carlsbad, CA, USA) as recommended by the manufacturers. Several isolates from each transformation were sequenced and analyzed as described above. Intron positions were identified by comparison with the cDNA sequence.

Identification of additional winter flounder pleurocidin-like sequences by RT-PCR

Total RNA was isolated from winter flounder skin and intestinesubstantially as described in Douglas, Gawlicka *et al* (1999). Reverse transcription of 2 μg of total

RNA was performed using the RETROScript kit (Ambion, Austin, TX, USA) according to the manufacturer's recommendation. PCR was performed using PL3' and a primer corresponding to the amino terminus of the precursor polypeptide (PL5'; Table 1). The amplification conditions were: 1 min at 94° C; 32 cycles of 30 s at 94° C, 30 s at 50° C, 90 s at 72° C; and 2 min at 72° C and products were resolved on a 2% NuSeive gel. Bands were excised, cloned and sequenced as described above.

Identification of additional pleurocidin-like sequences from different tissues

Tissue-specific expression of pleurocidin was investigated by northern analysis using polyadenylated RNA (500 ng) from adult skin, liver, ovary, muscle, spleen, pyloric caeca, stomach and intestine. The entire insert from the cDNA clone corresponding to WF2 was radioactively labelled and incubated with the blot overnight at 60° C in UltraHyb hybridisation solution (Ambion, Austin, TX, USA). The blot was washed to a stringency of 50° C in 1X SSC/0.1% SDS for 1 h before exposure to X-ray film. RT-PCR was also employed using primers specific to WF1, WF1a, WF2, WF3, WF4, WFYT and WFX (Table 2) to assay expression of the different pleurocidin-like variants in various tissues. The conditions used were as described in the preceding paragraph except that the annealing temperature was 52 ° C.

Identification of additional pleurocidin-like sequences from different developmental stages

Two larval time series were used to assess developmental expression of pleurocidin-like genes. In the first, RNA was isolated from pooled samples of twenty whole larvae (5 and 13 dph), ten whole metamorphosing larvae (20 dph) and newly metamorphosed larvae (27 dph), gut tissue of two juveniles (41 dph), skin from the upper and lower side of adult fish and tissue from adult upper and lower intestine. RNA was isolated as described (Douglas, Gawlicka et al. 1999), the disclosure of which is incorporated herein by reference, and the assays were performed using the primers PL5' and PL2 and conditions described above for RT-PCR. Amplification of the actin mRNA was performed as previously described (Douglas, Bullerwell et al. 1999), the disclosure of which is incorporated herein by reference, to confirm the steady level of expression of a housekeeping gene and to provide an internal control for pleurocidin expression. In the second larval time series, RNA was isolated from

pooled samples of twenty whole larvae (hatch, 5 and 9 dph), ten whole larvae (15, 20, 25, 30 and 36 dph) and gut tissue of two juveniles (41 dph). Assays were performed using primers specific to WF1, WF1a, WF2, WF3, WF4, WFYT and WFX (Table 2) to determine expression of the different pleurocidin-like variants at different stages of development. The conditions used were as described in the preceding paragraph.

Southern analysis

Southern analysis of *Bam*HI- and *Sst*I-digested genomic DNA from winter flounder, three other flatfish (American plaice *Hippoglossoides platessoides* Fabricius, Atlantic halibut *Hippoglossus hippoglossus* L. and yellowtail flounder *Pleuronectes ferruginea* Storer), haddock (*Melanogrammus aeglefinus* L.), pollock (*Pollachius virens* L.) and smelt (*Osmerus mordax* Mitchill) was performed sequentially using the entire inserts from genomic clones corresponding to WF1, WF2, WF3 and WF4 as probes. Hybridisations were performed overnight at 65° C as previously described (Douglas, Gallant et al. 1998), the disclosure of which is incorporated herein by reference, and the blots were washed at 65° C in 0.5X SSC/0.1% SDS for 1 h and exposed to X-ray film. Blots were stripped by incubating twice in boiling 0.5% SDS and checked for residual signal by exposure to X-ray film overnight.

Identification of additional pleurocidin-like sequences from other fish species

Total RNA was isolated from skin and intestine of yellowtail flounder, witch flounder and Atlantic halibut and reverse-transcribed as described above (RT-PCR analysis). Total genomic DNA was isolated from milt of yellowtail flounder, witch flounder, American plaice, Atlantic halibut and tissue samples of Petrale sole, C-O sole, English sole, Starry flounder, European plaice, Greenland halibut and Pacific halibut. Two sets of primers specific to the winter flounder pleurocidin cDNA (PL1/PL2 and PL5'/PL3'; Table 1; Fig. 1) were used and the amplification conditions were: 1 min at 94° C, 32 cycles of 30 s at 94° C; 30 s at 50° C, 90 s at 72° C; and 2 min at 72° C. Products were resolved on a 2% NuSeive gel, bands excised, cloned and sequenced as described above.

Figure 1 is a textual and graphical depiction of WF2 pleurocidin from winter flounder A. Nucleotide sequence of cDNA for pleurocidin from winter flounder isolated from the skin library. The positions of primers used for PCR are underlined and the deduced amino acid sequence is shown in upper case letters below the nucleotide sequence. Arrows indicate the mature 5' and 3' termini of the pleurocidin peptide and diamonds indicate the positions of introns. The single *SstI* restriction endonuclease site (GAGCTC) and the putative polyadenylation site (aataaa) are indicated in boldface. *B.* Hydrophobicity plot of predicted pleurocidin polypeptide WF2 constructed using the Kyte-Doolittle option of DNA Strider (Marck 1992). The borders of the mature pleurocidin are indicated by vertical arrows. *C.* Diagrammatic representation of helical structure of predicted pleurocidin polypeptide WF2 constructed using the Helical Wheel routine of GCG. Hydrophobic residues and glycines are boxed and polar residues are not. The first amino acid (G) of the mature polypeptide is found at the top of the wheel.

Identification of pleurocidin-like sequences in the winter flounder genome

A winter flounder genomic λ-GEM library was screened using a radioactively labeled probe for pleurocidin (WF2; Douglas et al., 2001). Four clones were picked and replated until 100% purity was achieved. The clones were mapped using *BamHI, SstI, XhoI* and *Eco RI* and two clones (λ1.1 and λ5.1) that differed in restriction pattern were selected for sequencing. Both clones were completely sequenced using an ABI373 stretch automated sequencer and the AmpliTaqFS Dye Terminator Cycle Sequencing Ready Reaction kit (Perkin Elmer, Foster City, CA, USA. Transcription factor binding sites were identified using WWW Signal Scan (http://bimas.dcrt.nih.gov/molbio/signal/) with the TransFac and TFD databases and promoters were detected using the eukaryotic promoter prediction by neural network software available at the Baylor College of Medicine (http://searchlauncher.bcm.tmc.edu/seq-search/gene-search.html).

Hepcidins

The general approach followed is depicted in Figure 24

Molecular Characterisation of Hepcidin cDNAs

Eight ESTs showing high similarity to human hepcidin were identified from the winter flounder EST database (Douglas, Gallant et al. 1999) and four from the Atlantic salmon database (Douglas, Tsoi et al. 2002). Using these sequences to screen dbEST, BLASTX analysis revealed two related sequences from Japanese flounder (C23298.1 and C23432.1), one sequence from rainbow trout (AF281354_1) and five identical sequences from medaka (AU178966, AU179222, AU179314, AU179768 and AU180044). Sequence data were analyzed using Sequencher (Gene Codes, Inc., Ann Arbor, MI, USA) and DNA Strider (Marck 1992). Alignments and similarity matrices were calculated using ClustalW (Thompson, Higgins et al. 1994) and graphically visualised using SeqVu (Garvan 1996). The on-line servers PSORT (http://PSORT.nibb.ac.jp), Compute pI (http://expasy.hcuge.ch/cgi-bin/pi_tool), and Network Protein Sequence @nalysis (http://npsa-pbil.ibcp.fr/cgi-bin/secpred_consensus.pl) were used to predict N-terminal signal sequences, pI and secondary structure, respectively. The secondary structure prediction program utilized seven different algorithms (for details, see web site) and provided a consensus prediction based on these results.

Southern Hybridisation

Total genomic DNA was prepared from winter flounder (*Pleuronectes americanus*), yellowtail flounder (*Pleuronectes ferruginea*), witch flounder (*Glyptocephalus cynoglossus*), Japanese flounder (*Paralichthys olivaceus*), American plaice (*Hippoglossoides platessoides*), Atlantic salmon (*Salmo salar*), haddock (*Melanogrammus aeglefinus),* smelt (*Osmerus mordax*), hagfish (*Eptatretus burgeri*), tiger shark (*Scyliorhinus torazame*) and white sturgeon (*Acipenser transmontanus*) as previously described (Douglas, Bullerwell et al. 1999), the disclosure of which is incorporated herein by reference. DNA (7.5 □g) was digested with *SstI* according to the manufacturer's recommendations and the fragments resolved on a 1% agarose gel. A 104 bp probe corresponding to amino acid residues WMENPT. . . .GCGFCC (SEQ ID NO: 321 and 322 respectively) of Type I winter flounder hepcidin was labeled using the DIG Labelling Kit (Roche Applied Science, Laval, PQ, Canada) and hybridized to the membrane for 2h at 42 °C using the Easy Hyb kit (Roche Applied Science, Laval, PQ, Canada). The membrane was washed in 0.2X SSC at 65 °C and

signal detected using the DIG Luminescent Detection Kit (Roche Applied Science, Laval, PQ, Canada).

Identification of additional hepcidin-like sequences by RT-PCR

Primers were designed based on the cDNA sequences determined in this study (Table 3). Amplification of actin mRNA was performed to confirm the steady-state level of expression of a housekeeping gene and provide an internal control for the hepcidin gene expression analyses. Controls were performed using single primers to eliminate single primer artifacts and without reverse transcription to eliminate amplification products arising from contaminating genomic DNA.

Total RNA was isolated from tissues of uninfected adult winter flounder and uninfected and infected adult salmon and halibut using the RNAWiz Kit (Ambion, Austin, TX, USA) according to the manufacturer's recommendations. Tissues were homogenized using a 7mm generator on a Polytron standard rotor stator homogenizer (Kinematica). In addition, RNA was isolated from pooled samples of twenty whole larvae (hatch, 5 and 9 dph), ten whole larvae (15, 20, 25, 30 and 36 dph), gut tissue of two juveniles (41 dph) and adult winter flounder liver. To eliminate contaminating DNA, the Ambion DNA-*free* TM protocol was used as directed. Briefly, 4 units of DNase 1 was added to the resuspended RNA and incubated for 1 hour at 37C. After incubation, DNAse Inactivation Reagent was added to remove the enzyme and RNA concentrations were determined using a Beckman DU-64 Spectrophotometer.

First strand cDNA was synthesized from 1 μg of total RNA using the RetroScript kit (Ambion, Austin, TX, USA) and aliquots of the reaction products were subjected to PCR using rTaq polymerase (Amersham Pharmacia Biotech AB, Uppsala, Sweden) or the Advantage2 PCR kit (Clontech, Palo Alto, CA, USA). The primers and annealing temperatures are listed in Table 3. The amplification conditions were: 1 min at 95° C; 32 cycles of 15 s at 95° C; 30 s at the annealing temperature, 30 s at 68° C; hold at 4° C. Amplification products were resolved on a 2% NuSieve agarose gel with a 100 bp ladder as a marker (Gibco BRL, Gaithersburg, MD, USA) and the amount of each product was quantified using a GelDoc 1000 video gel documentation system (BioRad, Mississauga, Ont., Canada) with the Multianalyst software.

Identification of additional hepcidin-like sequences from other fish species

Total RNA was isolated from liver and spleen of bacterially challenged Atlantic halibut and Atlantic salmon and reverse-transcribed as described above (RT-PCR analysis). Two sets of primers were used (see legend, Fig. 2) and the amplification conditions were: 2 min at 94° C; 32 cycles of 30 s at 94° C; 30 s at 52° C, 30 s at 72° C; and 2 min at 72° C. Products were resolved on a 2% NuSeive gel, bands excised, cloned and sequenced as described above.

Bacterial Strains and *Candida albicans*

All strains used in this study are listed in Table 5. Most non-fish bacterial strains as well as *Candida albicans* were grown at 37°C in Mueller-Hinton Broth (MHB; Difco Laboratories, Detroit), while the fish bacteria were maintained at 16°C in Tryptic Soy Broth (TSB; Difco, 5g/l NaCl). All strains were stored at –70°C until they were thawed for use and sub-cultured daily. The following strains, *Pseudomonas aeruginosa* K799 (parent of Z61), *Pseudomonas aeruginosa* Z61 (antibiotic supersusceptible), *Salmonella typhimurium* 14028s (parent of MS7953s), *Salmonella typhimurium* MS7953s (defensin supersusceptible), as well as *Staphylococcus epidermidis* (human clinical isolates) and methicillin-resistant *Staphylococcus aureus* (MRSA; isolated by Dr. A. Chow, University of British Columbia) have been kindly donated by Prof R.E.W. Hancock, University of British Columbia.

Escherichia coli strain CGSC 4908 (*his-67, thyA43, pyr-37*), auxotrophic for thymidine, uridine, and L-histidine (Cohen *et al.*, 1963) was kindly supplied, free of charge, by the *E.coli* Genetic Stock Centre (Yale University, New Haven, CT). MHB supplemented with 5 mg/L thymidine, 10 mg/L uridine and 20 mg/L L-histidine (Sigma Chemical Co., St. Louis, MO), was used to grow *E.coli* CGSC 4908 unless otherwise specified.

Two field isolates of the salmonid pathogen *Aeromonas salmonicida* are from the IMB strain collection.

Minimum Inhibitory Concentrations

The activities of the antimicrobial peptides were determined as minimal inhibitory concentrations (MICs) using the microtitre broth dilution method of Amsterdam (Amsterdam, 1996), as modified by Wu and Hancock (1999). Serial dilutions of the peptide were made in water in 96-well polypropylene (Costar, Corning Incorporated, Corning, New York) microtiter plates. Bacteria or *C. albicans* were grown overnight to mid-logarithmic phase as described above, and diluted to give a final inoculum size of 10^6 cfu/ml. A suspension of bacteria or yeast was added to each well of a 96 well plate and incubated overnight at the appropriate temperature. In the case of *E. coli* CGSC 4908, supplemented MHB was used. Inhibition was defined as growth lesser or equal to one-half of the growth observed in control wells, where no peptide was added. Three repeats of each MIC determination were performed.

Killing assays

Survival of bacteria and *C. albicans* upon exposure to selected peptides applied at their minimal inhibitory concentrations (MICs) and ten times their MICs was measured using standard methodology. The test organisms were grown in MHB and exposed to the peptides. At the specified time intervals equal aliquots were removed from the cultures, plated on MHB plates, and the resulting colonies were counted. Percentage survival was plotted against time on a logarithmic scale. Two repeats of each experiment were performed.

Preparation of a Synthetic Antimicrobial Peptide

Prediction of active cationic peptide sequences.

The mature peptide sequences from Figure 3 (pleurocidin-like peptide sequences deduced from nucleotide sequences of genes and PCR products amplified from fish tissues) constituted the basis of sequence selection.

Upon extensive sequence analysis, sequences were selected for peptides that possessed a net positive charge and had their hydrophilic and hydrophobic residues well separated spatially in models.

Also, generally those peptide genes that were likely to be expressed (possessed promoters, were transcribed, etc.) were produced, although pseudogenes were also included in the panel.

The exact start/end residues were decided upon based on several factors:

a) In most cases the N-terminus of the mature peptide was well-defined, since it followed directly the conserved signal peptide region, and aligned well with other mature peptides.

b) Wherever a straightforward determination on the N-terminal amino acid was not possible, an attempt was made to preserve GW or GF at the N-terminus, as this is frequently encountered among cationic peptides.

c) In addition, two versions of WF1a (NRC-2 and NRC-3) were produced: one contained N-terminal GRRKRK (SEQ ID NO: 323), and the other did not; this was done because it was hypothesized that the presence of the highly positively charged GRRKRK (SEQ ID NO: 323) would improve activity.

d) Although in some cases the C-terminus of the mature peptide was also well defined, since it was followed directly by a conserved acidic propiece, significant ambiguity as to the C-terminal amino acid existed among many peptides. Generally, two rules were followed in deciding upon C-terminal amino acids:

1. wherever glycine appeared at or near the C-terminus, it was considered to be a precursor for carboxy – terminus amidation;

2. large numbers of negatively charged amino acids near the C-terminus were generally considered to be a part of the propiece and not mature active peptide and were not included in the sequence.

In order to estimate the net charge, K and R were assumed to have the value of +1, H of +1/2, D and E of -1, and C-terminal amidation was counted as an additional +1. The EMBOSS Pepwheel and Pepnet internet tools available through an NRC mirror site (http://bioinfo.pbi.nrc.ca:8090/EMBOSS/index.html) were used to analyse the

separation of hydrophilic and hydrophobic residues in helical wheel and helical net models.

All antimicrobial peptides used in this study were synthesized by N-(9-fluorenyl) methoxy carbonyl (Fmoc) chemistry at the Nucleic Acid Protein Service (NAPS) unit at the University of British Columbia. Peptide sequences are shown in Table 4. Peptide purity was confirmed by HPLC and mass spectrometry analysis in each case. In the case of NRC-7 further purification by RP-HPLC was performed until homogeneity of the sample was obtained.

Peptides produced according to the above steps are screened for antimicrobial activity *in vitro* by standard means. Those peptides showing *in vitro* antimicrobial activity are useful as antimicrobial peptides for use *in vivo* and for the treatment of surface, etc.

Examples - Results

Pleurocidins

cDNA sequence

The two clones isolated from the winter flounder skin cDNA library were identical in sequence to each other and to the genomic PCR product WF2after introns were removed (see below). They contain 356 bp and encode an open reading frame of 68 amino acids (Fig. 1A). There is a 5'-untranslated region of 26 bp and a 3'-untranslated region of 84 bp, excluding the polyA tail. A canonical polyadenylation signal AATAAA is found 22 bp upstream of the polyA tail. The first 22 amino acids of the open reading frame form a highly hydrophobic domain (Fig. 1B) predicted to be a signal peptide with a cleavage site that precisely matches the amino terminus of the mature pleurocidin. The predicted amino acid sequence of residues 23-47 exactly matches the published amino acid sequence of mature pleurocidin (arrows, Fig. 1A).

The mature peptide can assume an amphipathic helix that contains a predominance of positively charged amino acids on one face and hydrophobic amino acids on the other (Fig. 1C). The carboxy-terminal 21 amino acids form a negatively charged domain that is not present in the mature pleurocidin, confirming the recent report of Cole et al. (2000).

Genomic PCR

Four distinct bands (WF1-4) were amplified using primers PL5' and PL3' (Fig. 4). Sequence analysis of each product was consistent with the sizes of the bands and verified that each amplification product was different (Table 6). Two distinct bands were amplified using primers PL1 and PL2 that corresponded to WF2 and WF4 containing additional upstream and downstream sequence (data not shown). When the intron sequences were removed, the sequence of WF2 exactly matched that of the pleurocidin cDNA clone isolated from the skin library (Fig. 1A).

Figure 4 is a depiction of the results of PCR amplification of pleurocidin-like sequences from winter flounder genomic DNA. Amplification products (P) were resolved on a 1 % agarose gel using the 100 bp ladder as molecular weight markers (M). Products visible as distinct bands are labeled WF1 (00 bp), WF2 (810 bp), WF3 (650 bp) and WF4 (510 bp).

All four of the pleurocidin-like genes contained two introns within the coding sequence and three of the genes showed identical intron locations (WF1, WF2 and WF4). However, the position of the second intron in WF3 occurred upstream of those of the other genes, resulting in a shorter second exon and longer third exon. The sizes and sequences of the introns varied among the four pleurocidin genes (Table 6). Evidence from the two more extensive genomic sequences of WF2 and WF4 obtained using primers PL1 and PL2 indicates that a third intron immediately upstream of the initiation codon is also a feature of this gene family (Fig. 5). This was also noted for the genomic sequence reported by Cole et al (Cole, Darouiche et al. 2000).

An alignment of the predicted amino acid sequences is shown in Fig. 6. The positions of the introns (indicated by vertical arrows) were determined by comparison

with the corresponding RT-PCR and cDNA-derived sequences. The positions of the mature peptide were determined by comparison with the published amino acid sequence of pleurocidin (Cole, Weis et al. 1997). All of the predicted mature polypeptides could assume amphipathic α-helical structures similar to that shown in Fig. 1C, although the positively charged portions were not as striking in WF1 and WF3 as in WF2 and WF4 (data not shown).

Figure 5 describes extended genomic sequence of WF4 obtained by PCR using primers PL1/PL2. Introns are indicated in lower case and coding sequence in upper case The positions of the primers PL1 and PL2 used for PCR are underlined.

Figure 6 describes Alignment of predicted polypeptide sequences of five winter flounder pleurocidin family members. Large vertical arrows indicate the positions where introns were found in the genomic sequences. The second intron of WF3, indicated by a small vertical arrow, is found more upstream than those of the other genes. The predicted polypeptide sequences of dermaseptin B1 (Amiche et al. 1994) and ceratotoxin B (Marchini et al. 1995) are shown below the pleurocidin family members. Boxed amino acids are shared by half of the sequences.

Identification of additional pleurocidin-like sequences from different tissues

Northern analysis was only able to detect pleurocidin transcripts in skin (data not shown). However, the more sensitive RT-PCR assay indicated that pleurocidin was also expressed in other tissues, particularly gill and gut. Using primers PL5' and PL3', two bands were obtained from winter flounder skin (265 and 175 bp) and two from intestine (215 and 175 bp). Sequence analysis of several clones of each size showed that the 265 bp winter flounder skin clones corresponded to the genomic sequence of WF1 when intron sequences were removed (Table 7). Five of the 175 bp clones from skin and two of the 175 bp clones from intestine corresponded to the genomic sequence of WF2. This is consistent with results of northern analysis using the cDNA clone corresponding to the WF2 probe that showed hybridisation only to 200-nucleotide mRNA from the skin (data not shown). On the other hand, nine of the 175 bp clones from intestine and four of the 175 bp clones from skin corresponded to the genomic sequence of WF3. No RT-PCR products were obtained that corresponded to WF4. All seven of the 215 bp intestine clones corresponded to a novel family

member (WF1a) not represented by any of the winter flounder genomic sequences determined in this study.

Using primers specific to each of the pleurocidin-like variants reported above, as well as to additional pleurocidin-like variants identified on Lambda clones, we were able to demonstrate that different variants were expressed in different tissues (Fig. 7). WF2, WF3 and WFYT showed the expression in the widest distribution of tissues, whereas WF1 and WF4 were expressed in mainly in the gill and skin, and WFX was only expressed in the skin. Transcripts of WF1a could not be detected in any tissue.

Figure 7 describes the expression of specific pleurocidin-like genes in different tissues of winter flounder. Tissues were esophagus (E), pyloric stomach (PS), cardiac stomach (CS), pyloric caeca (PC), liver (L), spleen (SP), intestine (I), rectum (R), gill (G), brain (B) and skin (SK). Markers (M) were the 100 bp ladder. Primers were specific to each pleurocidin variant (Table 2)

Identification of additional pleurocidin-like sequences from different developmental stages

Using primers PL5' and PL2 (Table 1) from highly conserved regions of the pleurocidin-like peptides, low levels of transcripts were evident at 5 dph and increased during development (Fig. 8). Strong signals were obtained from adult skin and weak signals from intestinal tissue. Expression of the housekeeping gene, actin, was relatively constant throughout development.

Using primers specific to each of the pleurocidin-like variants reported above, as well as to additional pleurocidin-like variants identified on Lambda clones, it was demonstrated that different variants were expressed at different times during development (Fig. 9). WFX transcripts were only detectable at 20 dph, and WF2, WF3 and WFYT were detectable in premetamorphic larvae and metamorphic juveniles. No expression of WF1 and WF4 was detectable at any stage of development.

Figure 8 describes Reverse transcription-polymerase chain reaction assay of pleurocidin expression. Samples are from larvae (5 and 13 dph), metamorphosing

larvae (20 dph), newly metamorphosed larvae (27 dph), juveniles (41 dph), skin from the lower (LS) and upper side (US) of the fish and tissue from the lower (LI) and upper (UI) intestine. Primers specific for pleurocidin (panel A) and actin (panel B) were used.

Figure 9 describes Expression of specific pleurocidin-like genes during winter flounder larval development. Samples are from larvae (5, 9 and 15 dph), metamorphosing larvae (20 dph), newly metamorphosed larvae (25, 30 and 36 dph) and juveniles (41 dph). Controls using the 5' or 3' primers alone and with no template (NT) are also shown. Primers were specific to each pleurocidin variant (Table 2).

Southern analysis

Positive signals were specific to flatfish DNA using the WF1, WF2, WF3 and WF4 genomic probes (Fig. 10). No signals were detected with haddock, pollock or smelt DNA (data not shown). All four probes showed hybridisation to common *Sst*I and *BamHI* bands from the DNAs of all four flatfish, indicating that the genes are clustered on these genomes. The sizes of the hybridising fragments from the winter flounder digest are given in Table 8.

Figure 10 describes Southern analysis of pleurocidin genes of winter flounder (WF), yellowtail flounder (YF), American plaice (AP) and Atlantic halibut (AH). Total genomic DNA (7.5 μg) was digested with *Bam*HI (B) or *Sst*I (S) and the fragments resolved on a 1.0% agarose gel. The blot was hybridized successively with probes corresponding to WF1, WF2, WF3, and WF4. Markers (M) are lambda DNA digested with *Sty*I (24.0, 7.7, 6.2, 3.4, 2.7, 1.9, 1.4, 0.9 Kb).

Identification of additional pleurocidin-like sequences from other fish species

An alignment of the deduced amino acid sequences of pleurocidin-like peptides from American plaice, yellowtail flounder, witch flounder and Atlantic halibut is shown in Fig. 3. Sequences were obtained from genomic DNA of Petrale sole, C-O sole, English sole, starry flounder, European plaice, Greenland halibut and Pacific halibut. High conservation is present in the signal peptide and acidic propiece

regions, whereas the portion corresponding to the mature peptide shows much more variability.

Figure 3 describes Alignment of pleurocidin-like peptide sequences deduced from nucleotide sequences of genes and PCR products amplified from skin and/or intestine of the following species: winter flounder (WF), yellowtail flounder (YF), witch flounder (GC), American plaice (AP) and Atlantic halibut (AH). Specific non-limiting examples of pleurocidin-like sequences identified are shown in Table 4. Non-limiting examples of cDNA and/or genomic sequences are provided in Appendix I.

Identification of pleurocidin-like sequences in the winter flounder genome

Two clones containing fragments of 12.5 and 15.6 kb, respectively, were isolated from a genomic library from winter flounder. The 12.5 kb fragment encoded the gene corresponding to WF2 and two pseudogenes. The 15.6 kb fragment encoded the gene corresponding to WF1, one pseudogene and two previously undescribed pleurocidin-like sequences referred to as WFX and WFYT. A schematic of the gene arrangement is shown in Fig. 11. Scanning of the sequences upstream of the coding sequence revealed a canonical eukaryotic promoter, TATA and CAAT boxes as well as highly conserved sites for several transcriptions factors including NF-IL6, AP1 and α-interferon (Fig. 12). No promoter sequences were identified upstream of pseuodgenes.

Figure 12 describes Locations of transcription factor binding sites upstream of pleurocidin genes and pseudogenes. Promoters are indicated by hatched boxes, introns by solid boxes and genes and exons by stippled boxes.

Prediction and assessment of antimicrobially active peptide sequences

The minimal inhibitory concentrations of the chemically produced peptides against a wide range of baterial pathogens and *C. albicans* were determined and are shown in Table 9. Generally speaking many peptides showed the ability to inhibit the growth of a broad spectrum of bacterial pathogens and *C. albicans*. Particularly good examples of peptides with a broad spectrum of antimicrobial activity are the three peptides

derived from American plaice (NRC-11, NRC-12, and NRC-13) and three peptides derived from witch flounder (NRC-15, NRC-16, and NRC-17). Of those, NRC-15, NRC-13, and NRC-12 showed ability to kill methicillin-resistant *S. aureus* (Fig. 13), *P. aeruginosa* (Fig. 14) and *C. albicans* (Fig. 15), respectively.

Figure 13 describes Survival of a Gram–positive bacterium (methicillin-resistant *Staphylococcus aureus* - MRSA) upon exposure to NRC-15 at its minimal inhibitory concentration (MIC) and ten times its MIC. *S. aureus* was grown in Mueller-Hinton broth and exposed to NRC-15 at its MIC and ten times its MIC. At the specified intervals equal aliquots were removed from the culture, plated on MHB plates, and the resulting colonies were counted.

Figure 14 describes Survival of a Gram-negative bacterium (*Pseudomonas aeruginosa*) upon exposure to NRC-13 at its minimal inhibitory concentration (MIC) and ten times its MIC. *P. aeruginosa* was grown in Mueller-Hinton broth and exposed to NRC-13 at its MIC and ten times its MIC. At the specified intervals equal aliquots were removed from the culture, plated on MHB plates, and the resulting colonies were counted.

Figure 15 describes Survival of a yeast (*Candida albicans*) upon exposure to NRC-12 at its minimal inhibitory concentration (MIC) and ten times its MIC. *C. albicans* was grown in Mueller-Hinton broth and exposed to NRC-12 at its MIC and ten times its MIC. At the specified intervals equal aliquots were removed from the culture, plated on MHB plates, and the resulting colonies were counted.

In addition to demonstrating that pleurocidin-like peptides are active against a wide range of bacteria as well as *C. albicans*, the results indicate which factors should preferably be considered in selecting antimicrobially active peptides from genomic sequences.

Firstly, a notable group of peptides with poor or no observed activities were peptides derived from pseudogenes (NRC-8, NRC-9, NRC-10). These results indicate

that peptides capable of being expressed in the host organism may be better candidates for antimicrobials.

Secondly, the previously described N-terminal GRRKRK in WF1a (Fig. 2) proved to be a determinant of antimicrobial activity in NRC–3 as shown by the fact NRC-2 (identical to NRC-3 but missing the aforementioned fragment) was only marginally active (Table 9). This result stresses the importance of carefully selecting the start/end residues in the mature peptide, wherever these are not apparent in the original pre-pro-sequence.

Thus in an embodiment of the invention there is provided a group of pleurocidin-related antimicrobial peptides having the amino acid sequence GRRKRK. It will be appreciated that pleurocidin-like antimicrobial peptides lacking this sequence also exist and are specifically contemplated herein.

The previously described principles of: selecting positively charged peptides with good separation of hydrophilic and hydrophobic residues in helical wheel models, preserving GW or GF at the N-terminus, amidating the C-terminus where glycine was present, and cropping off clusters of acidic C-terminal amino acids were successful in selecting antimicrobially active peptides.

Peptides of the invention can be used at a range of pH's, salt concentrations, and temperatures. These peptides are useful against pathogens grown in biofilms or under any other conditions for pathogen growth or culture. See for example Figure 25 in which the ability of NRC-13 to kill *P. aeruginosa* K799 in 50 mM NaCl is shown. NRC-13 was added to a culture of *P. aeruginosa* supplemented with 150 mM NaCl to a final concentration of 4μg/ml (□) or 40 μg/ml (Δ), representing the MIC and 10X MIC, respetively. A control with no peptide added is also shown (♦).

Peptides may be used alone or in combination with one or both of their pre-and pro- sequences.

Peptides of the invention have many uses, including as antibacterial, antifungal, antiviral, anti-cancer, and antiparasitic agents, including in combination with other antibiotics, anti-infectives, and chemotherapeutants as well as with each other.

Peptides can be used as immunomodulatory agents such as for wound healing, tissue regeneration, anti-sepsis, immune promoters, etc. including in combination with other agents.

The peptides can be delivered topically (including e.g., aerosols-especially for respiratory tract infections in CF patients, ointments, lotions, rinses, eyewashes, etc.), systemically (including e.g. IV, IP, IM, subcutaneously, intracavity or transdermally) and, orally (e.g. pills, liquid medication, capsules, etc.).

Delivery via encapsulation, including in liposomes, proteinoids is contemplated, as is delivery in transgenic systems involving agricultural animals and/or plants.

Peptides can be used as protective coatings on medical devices (including catheters, etc, food preparation machinery and packaging.

Examples of antibiotics which can be used together with peptides disclosed herein in aquaculture operations include: Terramycin Aqua (oxytetracycline), Romet (sulfadimethoxine and ormetroprim), and Tribrissen (trimethoprim and sulfadiazine. In the hatchery, dipping in formaldehyde can be used together with peptides disclosed herein. Peptides can be used in combination with each other and/or in combination with conventional antibiotics for any of the uses described herein.

Bacterial Challenge

Three days post-injection, the infected Atlantic salmon were lethargic and anorexic. On sampling, the posterior kidneys of the injected fish were positive for *A. salmonicida* whereas those of the control fish were not.

Molecular Characterisation of Hepcidin cDNAs

Although the winter flounder EST database contains sequences from liver, ovary, stomach, intestine, spleen and pyloric caecae cDNA libraries and the Atlantic salmon

EST database contains sequences from liver, head kidney and spleen, hepcidin-like sequences were only detected in spleen and liver cDNA libraries of both fish. Four of 135 ESTs (3.0%) in the winter flounder liver library and two of 281 ESTs (0.7%) in the winter flounder spleen library encoded hepcidins. Three of 982 (0.3%) ESTs in the Atlantic salmon liver library encoded hepcidins. Five hepcidin sequences were also found in subtracted spleen (1.8%) and three in subtracted liver (0.6%) Atlantic salmon cDNA libraries that were enriched in transcripts up-regulated during infection with *Aeromonas salmonicida*. Unfortunately, since these are subtracted libraries, the inserts are only portions of the complete transcripts.

Analysis of the nucleotide sequences of Atlantic salmon hepcidin cDNAs revealed that one salmon EST (SL1-0412) was approximately 300 nucleotides longer than the other two. Furthermore, the hepcidin coding sequence was incomplete. Complete sequencing of this clone revealed the presence of two introns with standard GT/AG splice junctions (Fig. 16A). When removed, an open reading frame encoding a complete hepcidin-like peptide was obtained. Similarly, an incompletely spliced halibut transcript was amplified that still retained the second intron (Fig. 16B). Compared to mammals, the introns of salmon and probably halibut are in similar locations but of shorter length (Fig. 16C). In addition to these incompletely spliced cDNAs, we identified a winter flounder EST (WF4) that contains a large deletion relative to the other sequences that corresponded closely to the second exon of salmon and human hepcidin. Assuming the intron positions are conserved among vertebrates, this deletion could correspond to the removal of exon 2, and resulted in a peptide that differed from WF3a and WF3b in only five amino acid positions of the remaining peptide.

Figure 16 describes a Nucleotide sequence of unspliced liver cDNA encoding Type I salmonid hepcidin. Exon sequences are indicated in upper case letters and the deduced amino acid sequence is shown below the nucleotide sequence. The gt/ag intron/exon boundaries are highlighted in boldface and the polyadenylation signal (aataaa) is underlined. B. Nucleotide sequence of partially spliced cDNA from halibut spleen encoding Type I salmonid hepcidin. C. Comparison of intron/exon structure in human, mouse and salmon. Exons are represented by hatched boxes and introns by a single line (sizes in bp shown beneath).

The deduced amino acid sequences of five different winter flounder hepcidin cDNAs and two different Atlantic salmon hepcidins were aligned for comparison purposes with those extracted from dbEST corresponding to Japanese flounder (two), medaka (one) and rainbow trout (one), as well as the recently reported hepcidin from hybrid striped bass (Shike et al. 2002) and two from Atlantic halibut (Hb 17 and Hb 357). The sequences obtained from spleen and liver of Atlantic salmon (Sal2.1 and Sal8.6) and Atlantic halibut (Hb1.1, Hb5.3 and Hb7.5) by PCR are also included (Fig. 17). Human hepcidin was included as a representative of the mammals. The position of cleavage by signal peptidase was predicted by PSORT and the RX(K/)R motif typical of propeptide convertases (Nakayama 1997) was identified (vertical arrows; Fig. 17). The signal peptide sequence is 22-24 amino acids and is highly conserved among all of the fish sequences. The anionic propiece is 38-40 amino acids, depending on the particular hepcidin variant. The processed hepcidins contain 19-27 amino acids and all are positively charged at neutral pH except WF2 (Table 10). Types I and III hepcidin from flatfish as well as salmon type hepcidin contain eight cysteine residues in the mature peptide, which have been proposed to form four disulphide bonds. Type II winter flounder hepcidin is missing two cysteine residues, indicating that a maximum of three disulphide bonds could form. Hb357 contains only five cysteine residues and is quite different from the remaining hepcidin-like sequences. Results of secondary structure prediction methods indicated that the consensus structure of fish hepcidins was mostly random coil, although short stretches of extended strand were predicted by some methods.

Figure 17 describes Alignment of winter flounder (WF1, WF2, WF3a, WF3b, WF4), Atlantic halibut (Hb1.1, Hb5.3, Hb7.5, Hb17, Hb357) and Atlantic salmon (Sal1, Sal2, Sal2.1, Sal8.6) hepcidins with those of Japanese flounder (JFL4, JFL6), medaka, hybrid striped bass and human. A partial sequence from rainbow trout (GenBank accession AF281354_1) is also shown. The predicted positions of signal peptidase and pre-protein cleavages are indicated by arrows.

From Figure 17, it is apparent that all of the flatfish-type hepcidins have very similar signal peptides, which differ somewhat from the salmonid type and human hepcidin. Other novel features identified included different groups of hepcidins based

on (1) number of cysteines, (2) unique insertion FKC in flatfish Type III, (3) two other locations that may contain unique insertions (4) a truncated version (Flatfish Type IV), (5) longer versions at the amino terminus.

Based on the alignment, it is apparent that there are at least three different groups of flatfish hepcidins distinguishable by shared insertions and deletions. WF2 and JFL6 (Flatfish Type II) share a deletion of seven amino acids near the KR cleavage site resulting in a processed peptide of 19 amino acids, whereas WF3a, WF3b, WF4, Hb1.1, Hb17, Hb5.3 and Sal8.6 (Flatfish Type III) exhibit a deletion of only four amino acids (excluding the portion corresponding to the missing exon of WF4) resulting in processed peptides of 22 amino acids. WF1 and JFL4 (Flatfish Type I) do not contain this deletion but do contain an insertion relative to all other reported hepcidins at a position adjacent to the signal peptidase cleavage site. In addition, WF1, bass and medaka share an insertion of one amino acid within the mature peptide relative to all other reported hepcidins, giving a peptide of 26-27 amino acids. WF3a and WF3b differ from each other by only one amino acid although they contain several silent substitutions and differences in the 5' and 3' untranslated regions. Hb357 represents a possible fourth class of flatfish hepcidins. The 3' untranslated regions of WF2 and WF1 are very different from those of the other hepcidin transcripts, WF2 containing a long additional portion relative to the others and WF1being shorter and less highly conserved (Fig. 18A).

The salmonid hepcidin-like peptides fall into one group; the four reported sequences all share two deletions and differ from each other by four amino acids in the mature peptide and four amino acids in the upstream pre-protein portion. The 3' untranslated regions of the salmon hepcidins are only moderately conserved (Fig. 18B).

Figure 18 describes Alignment of 3' untranslated regions of (A) winter flounder (WF1, WF2, WF3a, WF3b, WF4) and (B) Atlantic salmon (Sal1, Sal2) hepcidin cDNAs. Conserved nucleotides are boxed. The positions of the primers used to amplify hepcidin homologs from halibut and salmon are indicated by arrows.

Genomic Organisation of Winter Flounder Hepcidin Genes

Southern hybridization analysis of genomic DNA from a wide variety of fish with a probe corresponding to Type I hepcidin identified bands in all flatfish tested but none of the other fish species (Fig. 19). In winter flounder, two fragments of 4.3 and 4.5 kb hybridized with the probe. Two fragments of yellowtail flounder of identical size hybridized (4.3 kb) and two fragments of witch flounder genomic DNA also hybridized (4.3 and 20 kb), whereas only one fragment (4.3 kb) of the American plaice and one fragment (5.5kb) of the Japanese flounder genomic DNA hybridized.

Figure 19 describes Southern hybridization analysis of hepcidin in different fish species. *Sst*I digests of genomic DNA (7.5 μg) from hagfish (Hg), shark (Sh), white sturgeon (St), winter flounder (WF), yellowtail flounder (YF), American plaice (AP), witch flounder (Wi), Japanese flounder (JF), Atlantic salmon (AS), smelt (Sm) and haddock (Hd) were hybridized with Type I hepcidin from winter flounder. Size markers (M) are Lambda DNA digested with *StyI*.

Identification of Hepcidin-like sequences by RT-PCR

Figure 2 describes amplification of hepcidin cDNAs from halibut and salmon liver and spleen. RNA was prepared from tissues of fish infected with a bacterial pathogen to induce expression of antimicrobial peptide genes, reverse-transcribed and subjected to PCR using the primers listed below. Actin was run as a control to show expression of a house-keeping gene. The labelling on the figure is as follows: HL - halibut liver; SL - salmon liver; HS - halibut spleen; SS - salmon spleen; M – markers. For the primers 5'U is the Universal 5' primer used in all reactions, Sal is Hc Sal (below) and WF is HcPA3b (below).

HepUniversal 5': AAGATGAAGACATTCAGTGTTGCA (SEQ ID NO: 324)

HcPA3 3'B2: GTTGTTGGAGCAGGAATCC (SEQ ID NO: 325)

Hc Sal: TGCTGGCAGGTCCTCAGAATTTGC (SEQ ID NO: 326)

The results of RT-PCR assays of tissue-specific expression of the three winter flounder hepcidins are shown in Fig. 20. Type I hepcidin was abundantly expressed in the liver and, to a lesser extent, in the cardiac stomach. Type II hepcidin could not be

detected in any tissues, whereas Type III hepcidin was moderately expressed in the esophagus, cardiac stomach, and liver.

In uninfected Atlantic salmon, Type I hepcidin was expressed at quite high levels in the liver, blood and muscle, at low levels in gill and skin, and at barely detectable levels in anterior and posterior kidney (Fig. 21A, Table 10). Type II hepcidin was expressed at barely detectable levels in the gill and skin only (Fig. 21B). However, fish infected with *Aeromonas salmonicida* showed expression of both types of hepcidin in most tissues tested (see below).

RT-PCR analysis of hepcidin gene expression in winter flounder larvae of different ages is shown in Fig. 22. Transcripts of Type II hepcidins could not be detected at any stage of development, whereas Type I and Type III hepcidins were detectable in pre-metamorphic larvae. Type I hepcidin was more abundantly expressed than Type II hepcidin and was also expressed at an earlier time (5 dph vs. 9 dph.).

Figure 20 describes Reverse transcription-PCR assay of hepcidin and actin gene expression in different tissues of winter flounder. Amplification products from adult winter flounder were amplified using gene-specific primers for Flatfish Type I (panel A), Type II (panel B) and Type III (panel C) hepcidins and for actin (310 bp) and resolved by electrophoresis on a 2% agarose gel. Markers (M) are the 100 bp ladder (BRL)

Figure 21 describes Reverse transcription-PCR assay of hepcidin and actin gene expression in different tissues of control Atlantic salmon (C) and those infected with *Aeromonas salmonicida* (I). Amplification products from reactions using gene-specific primers for Salmonid Type I (panel A) and Type II (panel B) hepcidins (163 bp) and for actin (400 bp) were resolved by electrophoresis on a 2% agarose gel. Markers (M) are the 100 bp ladder (BRL).

Figure 22 describes Reverse transcription-PCR assay of hepcidin and actin expression in developing winter flounder larvae. Samples were larvae at 5 dph (lane 1), 12 dph (lane 2), 19 dph (lane 3), 27 dph (lane 4), 41 dph (lane 5) and adult (lane 6). Amplification products from reactions using gene-specific primers for Flatfish Type I (panel A), Type II (panel B) and Type III (panel C) hepcidins and for actin

(400 bp) were resolved by electrophoresis on a 2% agarose gel using a 100 bp ladder (Pharmacia) as markers (lane M).

Identification of additional hepcidin-like sequences from other fish species

Using a primer based on highly conserved sequences in the signal peptide of all reported hepcidins (Hep Universal 5') in combination with primers based on highly conserved sequences in the 3' UTR of salmon (HcSal 3') and flatfish (HcPA3b 3'), it was possible to amplify hepcidin-like sequences from the liver and spleen of halibut and salmon (Fig. 2). An alignment of the deduced amino acid sequences of hepcidin-like peptides from winter flounder, Atlantic halibut and Atlantic salmon is shown in Fig. 17. Interestingly, flatfish-type hepcidin could be amplified from salmon (S8.6) and salmon-type hepcidin could also be amplified from a flatfish (Hb7.5). Additonal sequences were obtained from genomic DNA of Petrale sole, C-O sole, English sole, starry flounder, European plaice, Greenland halibut and Pacific halibut.

Figure 17 depicts an alignment of certain winter flounder (WF1, WF2, WF3a, WF3b, WF4) Atlantic halibut (Hb1.1, Hb5.3, Hb7.5, Hb17, Hb357) and Atlantic salmon (Sal1, Sal2, Sal2.1, Sal8.6) hepcidins with those of Japanese flounder (JFL4, JFL6, medaka, hybrid striped bass and human. A partial sequence from rainbow trout (Genbank Accession AF281354_1) is also shown. The predicted positions of signal peptidase and pre-protein cleavages are indicated by arrows.

Specific non-limiting examples of hepcidin sequences identified are shown in Table 11. Examples of cDNA or genomic sequences are shown in Table 13.

Pleurocidins

Most antimicrobial peptides, including cecropins and dermaseptins, are encoded by multigene families that have probably arisen by sequential gene duplications. We have demonstrated that the winter flounder, and probably other flatfish, possess a gene family encoding antimicrobial compounds similar to pleurocidin. Comparison of the genomic amplification products obtained using PL1/2 with the cDNA sequence (Fig. 1A) showed that WF2 and WF4 contain three introns, the first of which occurs only 1 bp upstream from the initiator methionine. The second

and third introns both occur within the mature peptide. The genes for GLa, xenopsin, levitide and caerulein – all skin peptides from *Xenopus laevis* – also contain an intron 1 bp upstream from the initiator methionine (Kuchler et al 1989). The intron positions are conserved in all but WF3 (Fig. 6), but they differ dramatically in size (Table 5), indicating that a considerable period of evolutionary time has elapsed since the duplication events occurred, or that the intron sequences are relatively free to drift.

Southern analysis shows that WF1-4 probes hybridise to other flatfish DNAs, including yellowtail flounder, Atlantic halibut and American plaice, but not to haddock, smelt or pollock. This hybridisation could be due to the highly conserved signal sequence and anionic portion which we have shown to be conserved in sequences isolated from these flatfish. Flatfish may provide a rich reservoir of potential therapeutants for the aquaculture industry. The probes for the different pleurocidin family members often recognise the same restriction fragments in winter flounder DNA, indicating that they may be clustered at a single locus on the genome. Complete sequencing of two Lambda clones hybridizing to pleurocidin confirms that such clustering does in fact occur (Fig. 11). Clustering of antimicrobial peptide genes has also been noted for insect cecropins (Gudmundson et al. 1991) and apidaecins (Casteels-Jossen et al. 1993), among others.

Figure 11 describes an embodiment of a Schematic of genomic organization of pleurocidin-like genes and pseudogenes (ψ) from winter flounder. Introns are represented by solid boxes and exons by stippled boxes.

All of the members of the pleurocidin family are encoded as prepropolypeptides consisting of an amino-terminal signal sequence followed by the active peptide and ending with an acidic portion. The deduced amino acid sequences of the signal and acidic sequences are very highly conserved whereas those of the predicted mature antimicrobial peptides are more variable (Fig. 6). All, however, appear to fold into amphipathic α-helices. This sequence conservation has allowed us to use a genomic approach to identify many different members of the pleurocidin gene family, not only from winter flounder but also from a variety of other flatfish (Fig. 3, Table 4, Appendix I).

The structure of the pleurocidin prepro polypeptides bears certain resemblances to the frog dermaseptin precursors, which also contain a signal sequence of similar length (22 amino acids) and an acidic portion of 16-25 amino acids. From the full-length cDNA clone (Fig. 1A), the acidic portion of pleurocidin was shown to contain 21 residues. A major difference between the pleurocidin and dermaseptin prepolypeptides is the position of the acidic portion – downstream of the mature peptide in pleurocidin and upstream of the mature peptide in dermaseptins. The acidic proparts of defensins have been proposed to prevent interaction of the antimicrobial peptide with the membrane by neutralising the cationic charges (Valore et al. 1996) and this may also be its function in pleurocidin. This feature can be of practical significance for delivering peptides that are inactive until specifically cleaved.

The signal sequences and acidic carboxy-terminal sequences of the pleurocidin family members are extremely highly conserved. The former, and possibly the latter, are presumed to target the precursor molecules to the cell membrane for secretion. Gene families for antimicrobial peptides that contain highly conserved signal peptides (often encoded by the first exon) followed by end products with different biological activities have been described from the dermaseptin family (Valore et al. 1996) and the GLa, xenopsin, levitide and caerulein, all of which are skin peptides from *Xenopus laevis* (Kuchler et al. 1989). These authors proposed that this modular gene structure allows targeting for secretion to be achieved for markedly different peptides using a common pathway. In the pleurocidin gene family, a modular structure is also present with exon 2 encoding the signal sequence and first half of the antimicrobial peptide, exon 3 encoding the next ten amino acids of the antimicrobial peptide, and exon 4 encoding the last three amino acids of the antimicrobial peptide and the acidic carboxy terminus.

The mature peptides encoded by WF2 and WF4 are 60% identical to each other (Fig. 6) and somewhat less similar to dermaseptin B1 and ceratotoxin B (Cole et al. 1997). WF1 is 64% identical to WF1a but contains a remarkably cationic stretch of 18 amino acids between the signal sequence and the mature peptide that is not present in WF1a. Whether or not this potentially antimicrobial 18-mer peptide arises when pleurocidin WF1 processing occurs remains to be determined. Both WF1 and WF1a

contain an additional 10-11 amino acids relative WF2, WF3 and WF4 between the mature peptide and the acidic carboxy terminus. WF3 shares similarities with both WF2/4 and WF1/1a. Synthetic pleurocidin identical to the central portion of WF2 has been shown to protect Coho salmon against infection by *Vibrio anguillarum*, as have hybrid peptides based on pleurocidin, dermaseptin and ceratotoxin (Jia et al. 2000).

The tissue-specific expression of the pleurocidin genes was assessed using northern blot analysis and RT-PCR. Northern analysis proved to be not sufficiently sensitive for detecting the low level of transcripts present in winter flounder mRNA. Transcripts were present only in skin in sufficient quantities to be detected by this method, so the more sensitive RT-PCR assay was used. Pleurocidin transcripts were found in both skin and intestine using this method, in agreement with the recently reported ultrastructural localisation of pleurocidin in these tissues (Cole, Darouiche et al. 2000) and supporting the role of pleurocidin in mucosal immunity. The transcript size (approximately 200 bp) is consistent with the size of products obtained by RT-PCR (Table 7), showing that the pleurocidin genes are transcribed separately.

RT-PCR analysis showed that the genes for the different pleurocidin-like peptides are expressed in a tissue-specific manner with WF2 being expressed predominantly in the skin and gill and to a lesser extent in the muscle, intestine, stomach and liver whereas WF1 and WF4 are detected predominantly in the gill and skin (Fig. 7). WF3 and WFYT are expressed in most of the tissues sampled, WFX is detected solely in the skin and WF1a was not expressed in any of the tissues sampled. Possibly, the different antimicrobial peptides are required to control the growth of different bacterial populations in the two tissues. Since no RT-PCR products were detected for WF4, it is possible that this gene is expressed only at low levels in adult skin or intestine or that it is expressed at a different life stage or in a different tissue.

Using primers that did not discriminate between the transcripts of the various pleurocidin-like genes, expression was first detected at 5 dph and showed a progressive increase towards adulthood. However, recent experiments using primers specific for WF1, WF1a, WF2, WF3, WF4 , WFX and WFYT, transcripts were detected at different developmental stages (Fig. 9). WFX was only detectable at 20 dph, whereas WFYT, WF3 and WF2 were detectable at 5 dph and at higher levels between 25-36 dph. Interestingly, WF1 was not detectable at any larval stage and may

only be expressed under specific environmental conditions in response to specific bacterial pathogens, as has been shown for *Drosophila* (Rivas and Ganz 1999). This is the first demonstration of developmental expression of an antimicrobial peptide in fish and shows that at least this component of innate immunity is present in early larval stages of winter flounder. Larval mortality prior to metamorphosis is of great concern and although the reasons for such mortality are not yet known, high bacterial load in the gut has been proposed (Padros, Minkoff et al. 1993). The adaptive immune systems of flatfish have been shown to develop later than those of other teleosts (Padros, Sala et al. 1991). Thus, the ability of larvae to produce antimicrobial peptides during this period may be crucial to survival, and the identification of factors that increase the production of such compounds would be of great benefit to aquaculturalists.

These results of testing synthetic peptides against a variety of bacterial pathogens as well as the fungal pathogen, *Candida albicans*, show promising candidates with broad-spectrum antimicrobial activities. Of particular interest is the ability of the peptides NRC-13 and NRC-15 to inhibit the growth of methicillin-resistant *S. aureus* at concentrations as low as 4 μg/ml. NRC-13 is also capable of inhibiting the growth of *C. albicans* at 4 μg/ml, *P. aeruginosa* at 1 μg/ml (and killing *P. aeruginosa* at this concentration), and *A. salmonicida* at 2 μg/ml. This means that NRC-13 is highly active against a fish pathogen, a Gram-negative human bacterium, a drug-resistant Gram-positive human bacterium, and a yeast. The example of NRC-13 demonstrates the range of potential targets and applications for cationic antimicrobial peptides.

These results also validate the process we used for selecting antimicrobially active peptides from a large amount of sequence data. The ability to accurately predict which peptides are likely to be active is a crucial link between genomics and therapeutics. While much work remains to be done in this area, we have clearly demonstrated that judicious application of the principles described earlier will aid in selecting active peptides.

Thus, a variety of cDNA and genomic sequences encoding the precursors of antimicrobial peptides identical to or similar to pleurocidin from a variety of flatfish

species have been isolated. Northern hybridisation and sequence analysis of RT-PCR products showed that expression was tissue-specific. Most importantly, the timing of expression of different pleurocidin variants in developing larval winter flounder was determined, allowing an estimate of the onset of the innate immune system in this fish. These assays of pleurocidin expression are useful in directing the screening strategy for isolating novel peptide sequences expressed during specific tissues and/or developmental stages. Environmental parameters affecting the production of pleurocidin can also be assayed.

This work paves the way to further studies aimed at the over-expression of pleurocidin as a therapeutant for aquacultured fish and the production of disease-resistant fish through transgenic technology as has been demonstrated in transgenic tobacco expressing antimicrobial peptides (Jach et al. 1995) and proposed for fish (Jia et al. 2000). Furthermore, because many fish live in a saline environment, the properties of their antimicrobial peptides may be different from those produced by terrestrial animals and have application in unique situations. For instance, the pulmonary mucosa of patients with cystic fibrosis contain elevated NaCl concentrations, which inhibit the natural cationic peptides secreted by the lung (Goldman et al. 1997). Salt-adapted cationic peptides from marine fish may have application in the treatment of lung infections in these patients.

Hepcidins

Sequence analysis of one salmon EST (SL1-0412) and one halibut clone (Hb7.5), revealed the presence of unspliced transcripts and allowed the positions of some of the introns to be determined (Fig. 16). Similar to mouse, human and hybrid striped bass, the salmon hepcidin is composed of three exons and two introns (Park, Valore et al. 2001; Shike et al. 2002; Pigeon, Ilyin et al. 2001). The position of the first intron of salmon and bass are identical and correspond to a position two amino acids 5' to those of mouse and human. However, the second salmon intron and the second halibut intron of Hb7.5 correspond to a position two amino acids 3' to those of mouse and human and several amino acids 5' to that of the bass. This is probably due to "intron sliding" whereby the positions of introns have shifted by several nucleotides over the course of evolution. Interestingly, the deletion in WF4

corresponds precisely to the position of the first salmon intron and the second mouse/human intron, indicating an intermediate intron/exon structure.

Mouse contains two hepcidin genes that are clustered on the genome (Pigeon, Ilyin et al. 2001) but in human (Park, Valore et al. 2001) and striped bass (Shike et al. 2002) only one hepcidin gene has been identified. Although the number of hepcidin genes in winter flounder and Atlantic salmon remains to be determined, there are at least five in winter flounder, five in Atlantic halibut and four in Atlantic salmon. Since there are no *Sst*I sites within the hepcidin probe used in the Southern hybridization analysis, it is highly probable that the five winter flounder hepcidin genes reported here are clustered on two genomic fragments. Multiple genes for pleurocidin also exist (Douglas, Gallant et al. 2001) and are clustered on the genome (Fig. 11). Interestingly, all of the small flounders tested from the Atlantic exhibited a similar hybridizing band of 4.3 kb, indicating that they share similarity at the genomic level. Japanese flounder, found in the Pacific, exhibited a single hybridizing band of 5.5 kb.

The deduced amino acid sequences of the fish prepro-hepcidins can be aligned with those from mammals throughout their length but only show high similarity in the portion corresponding to the processed peptides (Fig. 17). However, within the fish, the signal peptide and the propiece are also very highly conserved. Conservation of these segments has also been noted in the pleurocidin family (Douglas, Gallant et al. 2001). The amino-termini of the processed peptides were assigned based on the amino acid sequence of human hepcidin (Krause, Neitz et al. 2000; Park, Valore et al. 2001) and the proximity to the RX(K/R)R motif characteristic of processing sites (Nakayama 1997). The molecular weights of the processed hepcidins from winter flounder and Atlantic salmon range from 1992 Da (WF2) to 3066 (WF1), comparable to hepcidins isolated from mouse, human and bass. With the exception of WF2, which has an acidic pI (5.54), the pIs of hepcidins are between 7.73 and 8.76.

Like pleurocidins, the amino acid sequences of the hepcidin variants are highly similar within species, suggesting relatively recent duplication of an ancestral gene. It is possible that the aquatic environment in which fish live necessitates the existence of a more diverse suite of antimicrobial peptides than in terrestrial

mammals. In addition, this component of the innate immune system plays a more major role in fish than in mammals, which have a more highly evolved adaptive immune system.

The human hepcidin molecule has been proposed to form a secondary structure containing a series of β-turns, loops and distorted β-sheets (Park, Valore et al. 2001). Consensus secondary structure prediction of fish hepcidins show that they contain mostly random coil structure with some extended strand structure. With the exception of WF2, JFL6 and Hb357, all hepcidins reported thus far contain eight cysteine residues which are proposed to form four disulphide bonds (Krause, Neitz et al. 2000; Park, Valore et al. 2001) in the following linkage pattern: 1-4, 2-8, 3-7, 5-6 (Park, Valore et al. 2001). The loss of cysteine residues 1 and 3 from WF2 suggests that at least one disulphide bond cannot form.

Using gene-specific primers, we were able to demonstrate that different hepcidin genes are expressed in different tissues of both winter flounder (Fig. 20) and Atlantic salmon (Fig. 21). In Atlantic salmon, hepcidin was detectable in normal uninfected fish predominantly in liver, blood and muscle (Type I) and to a lesser extent in gill and skin (both types). This is consistent with the presence of three ESTs for Type I hepcidin in cDNA libraries constructed from uninfected livers, and the absence of ESTs for Type II hepcidin in cDNA libraries constructed from uninfected liver, spleen and head kidney. Type II hepcidin expression appears be confined to external epithelial surfaces in contact with the aqueous environment, whereas Type I hepcidin expression is more widespread, being expressed in liver, blood and muscle as well as external epithelial surfaces. In uninfected winter flounder, no transcripts of Type II hepcidin could be detected in any tissue but transcripts of Types I and III hepcidin were present in the liver and cardiac stomach. Type III hepcidin transcripts were also present in the esophagus.

Mouse hepcidin was also reported to be predominantly expressed in liver, and weakly in stomach, intestine, colon, lungs, heart and thymus by Northern analysis using one of the mouse hepcidin sequences as probe (Pigeon, Ilyin et al. 2001). However, this study did not discriminate between the two hepcidin genes and it is not

known whether or not the two mouse genes are differentially expressed in tissues of mouse. Similarly, dot-blot analysis of human tissues and cell lines using the human hepcidin cDNA as probe revealed strong expression in adult and fetal liver and weaker expression in adult heart, fetal heart and adult spinal cord (Pigeon, Ilyin et al. 2001). An earlier study using RealTime quantitative RT-PCR (Krause, Neitz et al. 2000) revealed strong expression of hepcidin in human liver, heart and brain and weak expression in a variety of other tissues. Interestingly, we could not detect either Type I or Type II hepcidin expression in the brain of normal Atlantic salmon or winter flounder, or heart of normal Atlantic salmon. However, in infected animals, Type II hepcidin was expressed in both tissues, indicating that this form is the predominant one produced under conditions of stress.

It is intriguing that we detected transcripts of Type I hepcidin that were constitutively expressed in blood cells of Atlantic salmon. Constitutively expressed non-enzymic antimicrobial molecules have been reported only rarely in blood of fish; a small hydrophobic cationic peptide was found in mucus of rainbow trout (Smith et al., 2000) and moronecidin, an antimicrobial peptide from bass, was expressed in blood of uninfected animals (Lauth et al. 2002). Interestingly, expression of neither hepcidin increased in blood of infected salmon relative to the uninfected control animals. Possibly, hepcidin is fulfilling a role in iron homeostasis in control animals as well as an antimicrobial role. Its presence in circulating blood cells of uninfected animals may be a precautionary measure against impending infection.

Type I and II hepcidins from Atlantic salmon were up-regulated during infection with *Aeromonas salmonicida*, but to different extents in various tissues. While Type I hepcidin was noticeably up-regulated in the esophagus, stomach, pyloric caecae, liver, spleen, intestine, posterior kidney, rectum and muscle and to a lesser extent in anterior kidney and skin, Type II hepcidin showed a more dramatic increase in stomach, pyloric caecae, liver, spleen, intestine, brain, heart and muscle. Weaker up-regulation was present in esophagus, anterior and posterior kidney, skin and rectum. These results are consistent with those reported for bacterially challenged hybrid striped bass where up-regulation was most dramatic in liver, but was also demonstrated in skin, gill, intestine, spleen, anterior kidney and blood (Shike et al. 2002). It is not known whether there are multiple hepcidins in hybrid striped bass and,

if so, whether they are differentially expressed as in Atlantic salmon and winter flounder.

Studies with mice have shown a 4.3-fold increase in hepcidin expression in livers of mice injected with LPS and a 7-fold increase in primary hepatocytes exposed to LPS (Pigeon, Ilyin et al. 2001). These studies were based on Northern analysis using only one of the mouse hepcidin sequences as probe, and were therefore unable to distinguish possible differential expression of the two mouse variants. Similar increases were noted in livers of mice subjected to iron overload, but not for primary hepatocytes exposed to iron citrate, possibly due to the differentiation status of the cultured hepatocytes. The fact that both iron overload and LPS exposure increase hepcidin expression indicates the importance of these two factors in the host response to pathogens.

During infection, iron is removed from the system by various mechanisms so that it is unavailable for use by invading pathogens. It has been proposed that recently discovered transferrin receptor2 mediates iron uptake by hepatocytes and increases their expression of hepcidin (Fleming and Sly 2001; Nicolas, Bennoun et al. 2001). Hepcidin, in turn, increases iron accumulation in macrophages and increases dietary iron absorption in duodenal crypt cells via β2 microglobulin, HFE and transferrin receptor1. These crypt cells differentiate into enterocytes with reduced amounts of iron transport proteins, thereby decreasing dietary iron uptake. Hepcidin thus appears to play a crucial role in iron homeostasis during inflammation as well as acting as an antimicrobial peptide. It is also possible that hepcidin could modulate expression of liver-derived acute phase proteins and exhibit synergistic effects with other components of the immune system.

Antimicrobial peptides have been shown to modulate gene expression in mouse macrophages (Scott, Rosenberger et al. 2000), and it is possible that they may exert similar effects in fish macrophages or hepatocytes. The presence of a functional nuclear localization signal (four K/R residues in a row) within prohepcidin of mouse and human indicates that hepcidin could act as a signaling molecule involved in maintenance of iron homeostasis in these organisms (Pigeon, Ilyin et al. 2001).

Interestingly, the nuclear localization signal also contains the recognition signal for processing of prohepcidin, indicating that nuclear localization would occur only prior to removal of the propiece, or that the propiece itself is localized to the nucleus. Teleost hepcidins contain only 3 out of 4 K/R residues, which may not be sufficient for nuclear localization; a role for hepcidin in intracellular signaling awaits testing with synthetic or *in vitro*-expressed peptide.

In conclusion, the sequences of new hepcidin-like peptides from different fish species and the presence of related sequences in several flatfish species by Southern hybridization have been determined. Furthermore, it has been shown that the various types of fish hepcidins are differentially expressed in a tissue-specific manner in normal fish, as a result of bacterial infection, and during larval development, thus providing a strategy for identifying additional sequences for novel peptides. Apparently in fish, different tissues produce hepcidins in a constitutive or inducible manner, indicating that hepcidin variants may have different functions under different circumstances. Given their role in iron homeostasis in mammals, it is possible that fish hepcidin variants may fulfill this role as well as that of killing specific pathogens. *In vitro* expression of hepcidin variants will allow their spectrum of antimicrobial activity to be determined as well as their effect on the innate immune response.

Thus, there has been provided a method for identifying potential antimicrobial peptides.

Tables

Table 1. Nucleotide sequences of oligonucleotides used for isolating pleurocidin-like sequences (SEQ ID NOS: 1-10, left to right, in order of appearance)

Table 2. Nucleotide sequences of oligonucleotides used for assay of pleurocidin-like gene expression in different tissues and at different stages of development of winter flounder (SEQ ID NOS: 11-34, left to right, in order of appearance)

Table 3. Nucleotide sequences of primers used in RT-PCR assays to analyse hepcidin gene expression. (SEQ ID NOS: 35-61, left to right, in order of appearance) The amino acid sequence on which the 5' primer was based is shown. The 3' primers were within the 3' untranslated region (3' UTR). The annealing temperatures used in the PCR reactions and the sizes of the amplification products are listed.

Table 4. One-letter amino acid sequences for pleurocidins based on genomic and expression data (SEQ ID NOS: 62-81, respectively, in order of appearance)

Table 4a. Bacterial and *Candida* strains used in this study

Table 5. Sizes of introns (in bp) in genomic sequences amplified using primers PL5' and PL3'

Table 6. RT-PCR products from skin and intestine corresponding to different pleurocidin genes

Table 7. Sizes of bands (in kb) hybridising to pleurocidin probes in *Bam*HI and *Sst*I digests of winter flounder DNA

Table 8. Minimal inhibitory concentrations of pleurocidin-like cationic antimicrobial peptides against a wide spectrum of bacterial pathogens and *Candida albicans.*

Table 9. Characteristics of winter flounder and Atlantic salmon hepcidin-like peptides

Table 10. Results of PCR analysis of hepcidin expression

Table 11. One-letter amino acid sequences for certain hepcidins based on genomic and expression data, including NRC reference numbers (SEQ ID NOS: 174-211, respectively, in order of appearance)

Table 12. Nucleotide sequences of pleurocidin-like peptides of Table 4

Table 13. Nucleotide sequences of hepcidin-like peptides of Table 11.

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The mention of a reference is not an admission or suggestion that it is relevant to the patentability of anything disclosed herein.

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Table 1. Nucleotide sequences of oligonucleotides used for isolating pleurocidin-like sequences

Primer	Amino Acid Sequence	Nucleotide Sequence (5' ⇒ 3')
Screening cDNA library		
PleuroA	FFKKAAHVGKH	TTCTTCAAGAAGGCYGCYCAYGT[C/G]GG [C/A]AAGCA
PleuroB	HVGKAALTHYL[1]	CAYGT[C/G]GG[C/A]AAGGCYGCYCT[C/G] AA[C/T/A]CAYTACCT
Genomic PCR and RT-PCR		
PL1	5' untranslated	GCCCACTTTGTATTCGCAAG
PL2	3' untranslated	CTGAAGGCTCCTTCAAGGCG
PL5'	MKFTATF	ATGAAGTTCACTGCCACCTTC
PL3'	KRAVDE[1]	TCATCGACTGCGCGCTT

[1]complement

Table 2. Nucleotide sequences of oligonucleotides used for assay of pleurocidin-like gene expression in different tissues and at different stages of development of winter flounder

Gene	Primer	Amino Acid Sequence	Nucleotide Sequence (5' ⇒ 3')
WF1	RTWF1	KGRWLER	AAGGGCAGGTGGTTGGAAAGG
	RTWF1/3'	YQEGEE[1]	CCCTCCCCCTCCTGGTA
WF1a	RTWF1a	RKRKWLR	CGTAAGAGAAAGTGGTTGAGA
	RTWF1a/3'	YQEGEE[1]	CCCTCCCCCTCCTGGTA
WF2	RTWF2	KAAHVG	AAGGCTGCTCACGTTGGC
	PL2	3' untranslated	CTGAAGGCTCCTTCAAGGCG
WF3	RTWF3	FLGALIK	TTCTTAGGAGCCCTTATCAAA
	RTWF3/3'	YDEQQE[1]	CTCCTGCTGCTCGTCATA
WF4	RTWF4	HGRHAA	CATGGTCGTCATGCTGCC
	PL2	3' untranslated	CTGAAGGCTCCTTCAAGGCG
WFYT	RTWFYT	GFLFHG	GGGATTTCTTTTTCATGG
	RTWFYT/3'	SFDDNP[1]	GGGTTGTCATCGAATGAG
WFX	RTWFX	RSTEDI	CGTTCTACAGAGGACATC
	RTWFX/3'	DDDDSP[1]	GGGGCTGTCATCATCATC

Table 3. Nucleotide sequences of primers used in RT-PCR assays to analyse hepcidin gene expression. The amino acid sequence on which the 5' primer was based is shown. The 3' primers were within the 3' untranslated region (3' UTR). The annealing temperatures used in the PCR reactions and the sizes of the amplification products are listed.

Type	Primer	Amino acid	Nucleotide sequence	Annealing	Product
(size)		aequence	(5'⇒3')	temperature	size
(bp)					
Winter flounder					
Type I	HcPA1 5'	WMENPT	TGGATGGAGAATCCCACC	50°C	
137	HcPA1b 3'	3'UTR	GTGAGGTTGTGTTGCGGG		
Type II	HcPA2 5'	GMMPNN	GGGATGATGCCAAACAAC	50°C	
180	HcPA2b 3'	3' UTR	ACTTGGACTATGGGCTGAG		
Type III	HcPA3 5'	WMMPNN	TGGATGATGCCATACAAC	50°C	
118	HcPA3b 3'	3' UTR	GTTGTTGGAGCAGGAATCC		
Actin	ActF (WF)	AALVVD	TCGCTGCCCTCGTTGTTGAC	50°C	
312	ActR (WF)*	VLLTEAP*	GGAGCCTCGGTCAGCAGGA		
	ActinF1	VFPSIV	GTGTTCCATCCATCGTC	50°C	
194	Actin R1	HTFYNEL	GAGCTCGTTGTAGAAGGTGT		
Atlantic salmon					
Type I	HCSS 5'	MHLPEP	ATGCATCTGCCGGAGCCT	55°C	
163	Hep Liv R	3' UTR	CATTGCAAACATGTACAAACTAG		
Type II	Hep Sp F	MNLPMH	ATGAATCTGCCGATGCA	52°C	
163	Hep Sp R	3' UTR	GGGCAAATTAAAGGCG		
Actin	Act400F	IVGRPRHQ	TCGTCGGTCGTCCCAGGCATCAG	52°C	
400	Act400R	GYALPHAI	ATGGCGTGGGGCAGAGCGTAACC		

* complement

Table 4. Sequences of pleurocidin-like peptides used for activity testing. Final peptide sequences and patterns of C-terminal amidation were selected based on the analysis of translated nucleotide sequences and on principles described in the text.

Origin	Amino acid sequence	Code
Winter Flounder (1)	GKGRWLERIGKAGGIIIGGALDHL-NH_2	NRC-01[a]
Winter Flounder (1a)	WLRRIGKGVKIIGGAALDHL-NH_2	NRC-02[a, d]
Winter Flounder (1a-I)	GRRKRKWLRRIGKGVKIIGGAALDHL-NH_2	NRC-03[a, d]
Winter Flounder (2) 2.1	GWGSFFKKAAHVGKHVGKAALTHYL-NH_2	NRC-04[a]
Winter Flounder (3)	FLGALIKGAIHGGRFIHGMIQNHH-NH_2	NRC-05[a]
Winter Flounder (4) 1.1	GWGSIFKHGRHAAKHIGHAAVNHYL-NH_2	NRC-06[a]
Yellowtail Flounder YT2	RWGKWFKKATHVGKHVGKAALTAYL-NH_2	NRC-07[b]
Winter Flounder X	RSTEDIIKSISGGGFLNAMNA-NH_2	NRC-08[b,c]
Winter Flounder Y	FFRLLFHGVHHGGGYLNAA-NH_2	NRC-09[b.c]
Winter Flounder Z	FFRLLFHGVHHVGKIKPRA-NH_2	NRC-10[b,c]
American Plaice AP1	GWKSVFRKAKKVGKTVGGLALDHYL-NH_2	NRC-11[b]
American Plaice AP2	GWKKWFNRAKKVGKTVGGLAVDHYL-NH_2	NRC-12[b]
American Plaice AP3	GWRTLLKKAEVKTVGKLALKHYL-NH_2	NRC-13[b]
Witch Flounder GcSc4C5	AGWGSIFKHIFKAGKFIHGAIQAHND-NH_2	NRC-14[b]
Witch Flounder GcSc4B7	GFWGKLFKLGLHGIGLLHLHL-NH_2	NRC-15[b]
Witch Flounder GC3.8-t	GWKKWLRKGAKHLGQAAIK-NH_2	NRC-16[b]
Witch Flounder GC3.8	GWKKWLRKGAKHLGQAAIKGLAS	NRC-17[b]
Witch Flounder GC3.2	GWKKWFTKGERLSQRHFA	NRC-18[b]
Halibut Hb26	FLGLLFHGVHHVGKWIHGLIHGHH-NH_2	NRC-19[b]
Halibut Hb18	GFLGILFHGVHHGRKKALHMNSERRS	NRC-20[b]

[a] Peptide predicted from expressed tag and/or expression confirmed by RT-PCR and/or by *in situ* hybridization.
[b] Peptide predicted from genomic sequence
[c] Pseudogenes
[d] NRC-2 and NRC-3 are both derived from the same sequences with the latter including an additional N-terminal fragment.

Table 4a. Bacterial and *Candida* strains used in this study.

Species	Code ID	Comments
Escherichia coli	C498, UB1005	Parent of DC2
Escherichia coli	C500, DC2	Outer membrane-permeable mutant
Escherichia coli	C786, CGSC4908	Triple auxotroph (thy, uri, L-his)
Salmonella enterica s. Typhimurium	C587, 14028S	Parent of C610
Salmonella enterica s. Typhimurium	C610, MS4252S	Supersusceptible strain
Pseudomonas aeruginosa	H187, K799	Parent of H188
Pseudomonas aeruginosa	H188, Z61	Supersusceptible strain
Enterococcus faecalis	C625, ATCC29212	Standard strain (ATCC)
Staphylococcus aureus	C622, ATCC25923	Standard strain (ATCC)
Staphylococcus aureus	C623, SAP017	MRSA clinical isolate (from Tony Chow – VGH)
Staphylococcus epidermidis	C960, ATCC14990	Standard strain (ATCC)
Staphylococcus epidermidis	C621	Clinical isolate (from David Speert – Children's)
Bacillus subtilis	C971, ATCC6633	Standard strain (ATCC)
Aeromonsa salmonicida	99-1, A449	Field isolate being sequenced at IMB
Aeromonas salmonicida	97-4	Field isolate
Candida albicans	C627, CALB105	Yeast test strain

Table 5. Sizes of introns (in bp) in genomic sequences amplified using primers PL5' and PL3'

Gene	Exon 1 Total	Intron 1	Exon 2	Intron 2	Exon3	
WF1	154	539	31	95	82	901
WF1a[1]	103	?	31	?	82	?
WF2[2]	100	525	31	108	49	813
WF3	100	374	19	97	64	654
WF4[2]	100	230	31	101	49	511

[1]Intron sizes could not be determined as this sequence is only represented by an RT-PCR product

[2]Sequences were also amplified using primer PL1 and PL2

Table 6. RT-PCR products from skin and intestine corresponding to different pleurocidin genes

Skin	**Intestine**	**Size**	**Band**
4	n/d[1]	265bp	WF1
5	2	175bp	WF2
4	9	175bp	WF3
n/d[1]	n/d[1]	-	WF4
n/d[1]	7	215bp	n/d[2]

[1]not detected

[2] not detected by genomic PCR (corresponds to WF1a)

5

Table 7. Sizes of bands (in kb) hybridising to pleurocidin probes in *Bam*HI and *Sst*I digests of winter flounder DNA

Probe	*Bam*HI	*Sst*I
WF1	>24, 6	19, 17, 4.5, 4.4, 3.0, 2.9, 2.2, 1.3, x
WF2	6	19, 17, 4.5, 4.4, 2.9, x 1.3, x
WF3	>24	19, 17, 4.5, x 2.9, x 2.2, 1.3, x
WF4	17, 6	19, 17, 4.5, 4.4, 2.9, x 2.2, 1.3, 1.2

x=no hybridising band evident

Table 8. Minimal inhibitory concentrations of pleurocidin-like cationic antimicrobial peptides against a wide spectrum of bacterial pathogen and *Candida albicans*. Pathogens were grown in Mueller-Hinton broth and exposed to a range of concentrations of the

	A.sal 99-1	*A.sal* 97-4	*S.typh* MS4252 s	*S.typh* 14028s	*P.aeru* K799	*P.aeru* Z61	*E.coli* C786	*E.coli* UB1005	*E.coli* DC2	*S.epi* C621	MRSA C623	C.alb C627
NRC-1	64	64	16	>64	>64	32	32	32	32	>64	>64	64
NRC-2	>128	128	64	>64	64	32	64	64	64	>64	>64	>64
NRC-3	2	4	2	8	2	1	2	8	2	8	8	4
NRC-4	2	2	2	16	8	4	2	4	2	8	8	8
NRC-5	>64	>64	64	>64	>64	32	64	64	>64	32	32	>64
NRC-6	4	4	4	64	16	4	4	4	2	>64	32	32
NRC-7	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
NRC-8	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
NRC-9	>64	>64	64	>64	>64	64	64	>64	>64	>64	>64	>64
NRC-10	>64	32	16	>64	32	8	32	32	32	32	64	>64
NRC-11	8	8	4	32	32	4	4	16	4	64	>64	32
NRC-12	2	2	2	8	4	1	2	8	2	8	16	4
NRC-13	4	2	2	8	4	1	2	4	2	4	4	4
NRC-14	32	16	16	>64	32	8	16	16	16	16	16	>64
NRC-15	8	16	4	16	8	4	8	8	8	4	4	16
NRC-16	2	1	0.5	16	4	1	1	2	0.5	16	32	8
NRC-17	2	1	1	8	4	2	1	4	1	32	16	8
NRC-18	>64	128	32	>64	>64	64	64	64	64	>64	>64	>64
NRC-19	64	>64	16	64	32	8	32	16	32	8	8	64
NRC-20	>64	>64	>64	>64	>64	64	>64	>64	>64	>64	>64	>64

specified peptide. The lowest peptide concentration which inhibited bacterial growth by at least 50% was recorded as the minimal inhibitory concentration.

Table 9. Characteristics of winter flounder and Atlantic salmon hepcidin-like peptides

Name	Total Amino Acids	Total Cysteines	Molecular Weight	pI
WF1	27	8	3066	8.75
WF2	19	6	1992	5.54
WF3	22	8	2367	8.74
WF4	22	8	2256	8.52
Hb5.3	22	8	2363	8.75
Sal8.6	22	8	2331	8.76
Hb17	22	8	2391	8.76
Hb1.1	22	8	2391	8.76
Hb357	22	5	2397	7.84
Hb7.5	25	8	2881	8.53
Sal2.1	25	7	2925	8.60
Sal1	25	8	2720	7.73
Sal2	25	8	2881	8.53

Table 10. Semi-quantitative RT-PCR analysis of hepcidin expression in Atlantic salmon during bacterial challenge

Tissue	Type I Hepcidin			Type II Hepcidin		
	Control	Infected	Ratio	Control	Infected	Ratio
Esophagus	nd	0.08	↑	nd	0.09	↑
Stomach	nd	0.09	↑	nd	0.27	↑↑
Pyloric caecae	nd	0.14	↑	nd	0.37	↑↑
Liver	1.19	2.36	2	nd	1.45	↑↑↑
Spleen	nd	0.18	↑	nd	0.41	↑↑
Intestine	nd	0.21	↑	nd	0.33	↑↑
Brain	nd	nd	0	nd	0.50	↑↑
Blood	0.82	0.84	1	nd	nd	
Anterior kidney	0.06	0.07	1.2	nd	0.08	↑
Posterior kidney	0.07	0.14	2	nd	0.11	↑
Gill	0.13	0.12	1	0.08	0.07	1
Skin	0.14	0.18	1.3	0.07	0.09	1.3
Ovary	nd	nd	0	nd	nd	0
Rectum	0.07	0.13	2	nd	0.08	↑
Heart	nd	nd	0	nd	0.43	↑↑
Muscle	0.38	0.8	2.1	nd	0.60	↑↑

Pixel densities obtained by densitometry are expressed relative to the actin signal. The ratio of infected:control was calculated where numerical values were obtained for both conditions. nd, not detected; ↑ weakly up-regulated; ↑↑ strongly up-regulated.

Table 11. One-letter amino acid sequences for hepcidins based on genomic and expression data

Signal peptide	Anionic propiece	Mature peptide	NRC Code	Clone Name
<----------	---------->	<----------		
MKTFSVAVAVVVVLACMFILESTAVPFSEVRTEEVESIDSPVGEHQQPGGTSMNLPMHFRFKR-		QSHLSLCRWCCNCCHN-KGCGFCCKF	NRC201[b]	Hal7.5
MKTFSVAVAVVVVLACMFILESTAVPFSEVRTEEVESIDSPVGEHQQPGGTSMNLPMHFRFKR-		QSHLSLCRWCCNCCHN-KGCGFCCKF	NRC202[a]	Sal4.1
MKAFSVAVV--LVIACMFILESTAVPFSEVRTEEVGSFDSPVGEHQQPGGESMHLPEPFRFKR-		QIHLSLCGLCCNCCHN-IGCGFCCKF	NRC203[a]	Sal1
----------		RTEEVESIDSPVGEHQQPGGTSMNLPMHFRFKR-QSHLSLCRWCCNCCHN-KGCGFCCKF	NRC204[a]	Sal2
MKTFSVAVV--PVIACMFILESTAVPFSEVRTEEVGSFDSPVGEHQQPGGTSMNLPMHFRFKR-		QSHLSLCRWCFNCCHN-KGCGFCCKF	NRC205[a]	Sal2.1
MKQFSVAVV--LVMACMFIVESTAVPFSEVRTEEVGSLDSPVGEHQQPGGESMHLPEPFRFKR-		QIHLSLCGLCCNCCHN-IGCGFCCKF	NRC206[a]	Sal2.4
MKAFSIAVAVTLVLAFVCIQCSSAVPFQGVQELEEAGGNDTPVAEHQVMSMESWMENPTRQKRH		ISHISLCRWCCNCCKANKGCGFCCKF	NRC207[a]	WF1
MKTFSVAVAVTLVLAFVCIQDSSAVPFQGVQELEEAGGNDTPVAAHQMMSMESWMESPVRQKRH		ISHISM CRWCCNCCKA-KGCGPCCKF	NRC208[b]	JapF2.4
MKTFSVAVTVAVVLVFICIQQSSGTFPEVQELEEAVSNDNAAAEHQETSVDSWMMPYN-RQKR		**----AFKCKFCCGCCRA-GVCGLCCKF**	**NRC209[b]**	
MKTFSVAVTVAVVLVFICIQQSSASFPEAQELEEAVSNDNAAAEHQETPVDSWMMPYN-RQKR-		----SFKCKFCCGCCRA-GVCGLCCKF	NRC210[a]	WF3a
MKTFSVAVTVAVVLVFICIQQSSASFPEAQELEEAVSNDNAAAEHQETPVDSWMMPNN-RQKR-		----GFKCKFCCGCCRA-GVCGLCCKF	NRC211[a]	WF3b
MKTFSVAVTVAVVLVFICIQQSSATFPE----------		MPYN-RQKR----GFKCKFCCGCCGA-GVCGMCCKF	NRC212[a]	WF4
MKTFSVAVTVAVVLVFICIQQSSASFPEAQELEEAVSNDNAAAEHQETPVDS-RIPYN-RQKR-		----SFKCKFCCGCCRA-GVCGLCCKF	NRC213[b]	WFD1.1
MKTCSVAVTVAVVLVFICIQQSSASFPEVQELEEAVSNDNAAAEHQETPVDSWMMPNN-RQKR-		----GFKCKFCCGCCRA-GVCGLCCKF	NRC214[b]	WFD1.4
MKTISVAVTVAVVLVFICIQQSSASFPEAQELEEAVSNDNAAAEHQETPVDSGMIPYN-RQKR		**----SFKCKFCCGCCRA-GVCGLCCKF**	**NRC215[b]**	
MKTFSGAVTVAVVLVFICIQQSSASFPEVQELEEAVSNDNAAAEHQETPVDSWMMPNN-RQKR		**----GFKCKFCCGCCRA-GVCGLCCKF**	**NRC216[b]**	
MKTSVVAVTVAVVLVFICIQQSSATFPEVQELEEAVSNDNAAAAHQETSVDSWMMPYN-RPKR-		----SFKCKFCCGCCRA-GVCGLCCKF	NRC217 [b]	AP5.1
MKTFSVAVTVAVVLVFICIQQSSATFPEVQELEEAVSNDNAAAEHQETSVDSWMMPYN-RPKR-		----SFKCKFCCGCCRA-GVCGLCCKF	NRC218[b]	AP5.3
MKTFVVAVTVAVVLVFICIQQSSATFPEVQELEEAVSNDNAAAEHQETSVDSWMMPYN-RQKR-		----SFKCKFCCGCCRA-GVCGLCCKF	NRC219[b]	AP5.4
MKTSVVAVTVAVVLVFICIQQSSATFPEVQELEEAVSNDNAAAAHQETSVDSWMMPYN-RQKR-		----SFKCKFCCGCCRA-GVCGLCCKF	NRC220[b]	AP5.5
MKTFSVAVTVAVVLVFICIQQSSATFPEVQELEEAVSNDNAAAEHQETSVDLWMMPYN-RQKR-		----GFKCKFCCGCCSP-GVCGLCCRF	NRC221[b]	AP6.1
MKTFSVAVAVAVVLIFICIQQSSATFPEVQELEEAVSNDNAAAEHQETSLDSWMMPYN-RQKR-		----GFKCKFCCGCCRA-GVCGLCCKF	NRC222[b]	AP6.2
MKTFSVAVTVAVVLVFICIQQSSATFPEVQELEEAVSNDNAAAEHQETSLDSWMMPYN-RHKR-		----SFKCKFCCGCCRA-GVCGLCCKF	NRC223[b]	AP6.4

Table 11 (Cont.)

Signal peptide	Anionic propiece	Mature peptide	NRC Code	Clone Name
<---------->	<---------->	<---------->		
MKTFSVAVTVAVVLVFICIQQSSATFPEVQELGEAVSNDNAAAEHQETSVDSWMMPYN-	RPKR----	SFKCKFCCGCCRA-GVCGLCCKF	NRC224[b]	AP6.3
MKTFSVAVTVAVVLIFICIQQSSATSPEVQGLEEAVSNDNAAAEHQETSVDSWMMPYN-	RQKR----	GFKCKFCCGCCRP-GVCGLCCRS	NRC225[a]	Sal8.6
MKTFSVAVTVAVVLVFICIQQSSATFPEVQELEEAVSNDNAAAEHQETSVDLWMMPYN-	RQKR----	GFKCKFCCGCCRP-GVCGLCCRF	NRC226[b]	Hal7.1
MKTFSVAVTVAVVLVFICIQQSSATFPEVQELEEAVSNDNAAAEHQETSVDLWMMPYN-	RQKR----	GFKCKFCCGCCSP-GVCGLCCRF	NRC227[b]	Hal7.4
MKTFSVAVTVAVVLVFICIQQSSATFPEVQELEEAVSNDNAAAEHQETSVDSWMMPYN-	RQKR----	GFKCKFCCGCCSP-GVCGLCCKF	NRC228[b]	Hal8.2
MKTFSVAVTVAVVLVFICIQQSSATFPEVQELEEAVSNDNAAAEHQETSVDSWMMPYN-	RQKR----	GFKCKFCCGCCRP-GVCGLCCKF	NRC229[b]	Hal8.3
MKTFSVAVTVAVVLVFICIQQSSATFPEVQELEEAVSNDNAAAEHQETSVDSWMMPYN-	RQKR----	GFKCKFCCGCCRP-GVCGLCCKF	NRC230[a]	Hal5.3
MKTFSVAVTVAVVLVFICIQQSSATFPEVQELEEAVSNDNAAAEHQETSVDSWMMPYN-	RQKR----	GFKCKFCCGCCRP-GVCGLCCRF	NRC231[a]	Hal1.1
MKTFSVAVTVAVVLVFICIQQSSATFPEVQELEEAVSSDNAAAEHQETSVDSWMMPYN-	**RQKR----**	**SFKCKFCCGCCRR-GVCGLCCKF**	**NRC232[b]**	
MKTISVAVTVAVVLLFICTQQSSATFPEVQELEEAVSSDNAAAEHQETSVDSWMMPYN-	**RQKR----**	**GFKCKFCCGCRCG-ALCGLCCKF**	**NRC233[b]**	
MKTFSVAVTVAVVLVFICIQQSSATFPEVQELEEPVSSDNAAAEHQETSVDSWMMPYN-	**RQKR----**	**GFKCKFCCGCRCG-ALCGLCCKF**	**NRC234[b]**	
MKTFSVAVTVAVVLVFICIQQSSATFPEVQELEEAVSSDNAAAEHQETSVDSWMMPYN-	**RQKR----**	**GFKCKFCCGCRCG-ALCGLCCKF**	**NRC235[b]**	
MKTFSVAVTVAVVLVFICIQQSSATFPEVQELEEAVSNDNAAAEHQETPVDSGMMPNN-	RQKR----	SADCWPCCNQN----	GCGTCCKV	NRC236[a]	WF2
MKTFSVAVTVAVVLVFICIQQSSATFPEVQELEEAVSNDNAAAEHQETSVDSWMMPYN-	RQKR----	SAECSFCCNES----	GCGICCKF	NRC237[b]	YT11.1
MKTFSVAVTVAVVLVFICIQQSSATFPEVQELEEAVSNDNAAAEHQETSVDSWMMPYN-	RQKR----	SAECSFCCNES----	GCGICCKF	NRC238[b]	YT12.1
----------	----------	MPNN-	RQKR----	GSNCKPCCNHN----	GCGTCCEV	NRC239[b]	GC9.3

[a] Peptide predicted from expressed tag and/or RT-PCR product

[b] Peptide predicted from genomic sequence

- deletions introduced to clarify alignment

conserved cysteines are shaded

Table 12 Nucleotide sequences encoding pleurocidin-like peptides of Table 4.

NRC-01 Winter Flounder WF1 (SEQ ID NO: 82)
ATGAAGTTCACTGCCACCTTCCTCCTGTTGTTCATCTTCGTCCTCATGGTTGATCTCGGAGAGGGTCGTCGTAAGA
AAAAGGGGTCGAAGAGAAAGGGGTCCAAGGGAAAGGGGTCCAAGGGAAAGGGCAGGTGGTTGGAAAGGATTGGTAA
AGGTAGAGTCACGGAATTAATTTGCTTTTTACATTGCAAATATTTTTCATATAACATTGCTGGAAAATCACAAAAA
TAAGTAGTCAATATATTTGGCCAAATAGAATCACTTTGATTTCAATAATAATCAAAATAACAACCTAAAAGGCCTT
TGATTAGCATGTTCCTTCAATGAAATGGACATTGTAATTTACTTTGATTCTCACATGCTACGACCTGCTGCAGCAA
CATTTGAAAATAAATTTGTCCCAGAAGATTTTAAAGTACATTGTTATAGGCGATTTATCTTTCTATTACTCAGATA
TTTGTTCAAACCAATAGAATAACTGGATCTCTATGCTAAAATAATAAAACACACATTCAGATGTTACCAGTCAAGA
TTGAACGCTGTTTAAAAGTAAGTATGAAACATCCTCTGTATGTATAATTGTTTAACTGGTAACTTATAGTCCTAAT
AATTGCGTTATGGAAATGTATTAATTGTCATTTAATATAATTTGCTGGAATTTATCACTGTGTGTTTTTGTTTGTT
TTTACACAGCTGGCGGGATAATTATCGGGGGGGCCCTTGAGTAAGGACTTCTACCATCATTACTGTGTAATATTTA
TAGTTATGATCAGTACAGTTATTAACAACTTCTCTTGTCTCGCTGAACTTCTCCATCAGTCACCTCGGGCAGGGGC
AGGTGCAGGGGCCGGATTACGACTACCAGGAGGGGGAGGAGCTCAACAAGCGCGCAGTCGATGAA
//
NRC-02 and NRC-03 Winter Flounder WF1A (SEQ ID NO: 83)
ATGAAGTTCACTGCCACCTTCCTCCTGTTGTTCATCTTCGTCCTCATGGTTGATCTCGGAGAGGGTCGTCGTAAGA
GAAAGTGGTTGAGAAGGATTGGTAAAGGTGTCAAGATAATTGGCGGGGCGGCCCTTGATCACCTCGGGCAGGGGCA
GGTGCAGGGGCAGGATTACGACTACCAGGAGGGGCAGGAGCTCAACAAGCGCGCAGTCGATGAAA
//
NRC-04 Winter Flounder WF2 (SEQ ID NO: 84)
GCCCACTTTGTATTCGCAAGGTAATATTGATATTTTTCATATTCATTTAGACAAATGTGCTCAGCTTGTTACTGTA
TAATGCAAAAGTTAATGATCTTTATTTTTCTGTTTTTTTTTTGTAGAATGAAGTTCACTGCCACCTTCCTCATGATT
GCCATCTTCGTCCTCATGGTTGAACCTGGAGAGTGTGGCTGGGGAAGCTTTTTTAAAAAGGCTGCTCACGGTAGAG
TCACAGAATTAATTAGCTTTTTGCTTTGCAAATATTTTTTTTATAACAGCTGGAAAATCACAAAAATAAATAGTAT
ATATATTTGGCCAATAAAATCACTTTGATTTCAATAATAATCTAAATAACCAACCTAAAAGGCCTTTGATTAGCAT
GTTCCTTCAATGAAATGTACGTTGAGGTTTATTTTGATTCTCACAAGCACCAACCTGCTGCGTCAACAATTGAATT
CAAATTTGTCCCAAAGGAATTCAAAGTAAATTTTTCTAGGCGATTTAATCTTTCCATTACTCTGATTTGTTTTAAA
AATATAGAATAACTCAATCTCTATGATAAAACAATTACACATACATTCAGATTTTTATAGGACAAGATTGAAAACT
TCTTACAAGTATGTATAAAACATCATCTGTATGTATAATTGTTTAACATGTAACAACTAGTCCTACTAATTGTGTT
AAATTGTCATTTAATATCAATTGCTTGAGTTTATCATTATGTGTTTTGTTTTTTTTTACACAGTTGGCAAGCATGT
TGGCAAGGCGGCCCTTACGTAAGGACTTCTACCATTTTACTGTATAATTTTGATAGTGTTATCACCAGTACTGTTT
TTGACAACTTCTCTATTCCTGCTGACTCTCTCCATCCGACTCATCCGCAGTCATTACCTTGGCGATAAGCAGGAGC
TCAACAAGCGTGCAGTCGATGAAGACCCAAATGTTATTGTTTTTGAATGAAGAAAT
//
NRC-05 Winter Flounder WF3 (SEQ ID NO: 85)
ATGAAGTTCACTGCCACCTTCCTGGTGCTGTCCCTGGTCGTCCTAATGGCTGAGCCTGGAGAGTGTTTCTTAGGAG
CCCTTATCAAAGGGGCCATACATGGTAGAGTCAAGGAATTAATTAGATTTTTACATGTCAAATAATGTAGTAGAAC
ATATATAAGTAGTCAATATATTTGACCAAGTAGAATCATTTTGATTTCAATAATAATCAAAATAACAATCTCCAGG
CGATTTAATATTTGCAATAATTGGATTTTATAGAATACGGAACAACTGGATCTTAATGCTAAAATAATCCAACATA
CATTCTGATTTTGCCAGGCAAAATTAAACACTACTTTAAAGTATGTATAAAACATAATCTGTATGTTATAACAAAT
ACTCCAAGCAATTGTGTGATGGAAATGTATTCATTGTCATTTAATATAATTTGCTTGAGTTTATCATCTTGTGTTT
TTGTTTGTTTTTTCACAGGTGGCAGGTTTATCCATGGGTAAGGACTTCTACCATCATGACTGTGTATTTTTAATAT
TATTATCATCAGTACTGTTATTGACAACTTCACTTGTCTCGCTGACTCTCTCCATCAGAATGATCCAAAACCATCA
CGGTTATGACGAGCAGCAGGAGCTCAACAAGCGCGCAGTCGATGAA
//
NRC-06 Winter Flounder WF4 (SEQ ID NO: 86)
GCCCACTTTGTATTCGCAAGGTAATATCAATATTTTTCAAATTCATTTAGACGAGACCAACCTTTTGGGAAATCTG
CTCAGCTTATTACTGTATAATGCAAATGTTAATGATCTTTATTTTTCTGTTTTTTTTTTTGTAGAATGAAGTTCACT
GCCACCTTCCTCATGATGTTCATCTTCGTCCTCATGGTTGAACCTGGAGAGTGTGGTTGGGGAAGCATTTTTAAGC
ATGGTCGTCATGGTAAAGTCACGGAATTAATTAGCTTTTAACTTTGCAAATATTGTTTTTTTTTTTTAACAGCTGGA
AACTCACAAAAATAAATAGCCGATATATTTGGCCAATTATAATCACTTTGATCTAAATAACAACCTAAAAGGCCTT
TGATTAGCATGTTTCTTCAATAAAATGATTGAACACTACTTAAAGGTATGTATAAAACATCATCATGTGTTTTTGT
TTGTTTTTACACAGCTGCCAAGCATATTGGCCATGCAGCCGTTAAGTAAGGACTTCTACCATTATTACTGTATAAT
TTTGATAGTATTATCACCAGTATTGTTATTGACAACTTCTCTTTTTCCTGCTGATCCGACTCATCCGCAGTCATTA
CCTTGGCGAGCAGCAAGATCTCGACAAGCGCGCAGTCGATGAAGACCCAAATGTTATTGTTTTTGAATGAAGAAAT
//
NRC-07 Yellowtail Flounder YT2 (SEQ ID NO: 87)
ATGAAGTTCACTGCCACCTTCCTCATGATGTGCATCTTCGTCCTCATGGTTGAACCTGGAGAGTGTCGTTGGGGGA
AATGGTTTAAAAAGGCCACACACGGTAGAGTCACAGAATTAATTAGCTTTTTGCTTTGCAAATATTTTTTTATAAC
AGCTGGAAAATCACAAAAATAAATAGTCTATATATTTGGCCAATTAGAATCACTTTGCTTTCAATAAAAATCTAAA
TAACAACCTAAAAGTCCTTTGATTAGCATTTTCCATCAATGAAATGGACGTTGAGGTTTATTTTGATTCTCACATG
CACCGACCTGCTATGTCAACAATTGAATACAAATTTGTCCCAGAGGAATTCAAAGGAAATTTTTCTAGGCGATCTA

ATCTTTCCATTACTCGGATTTGTTTTTAAATATATAGAATAACTCAATCTCTATGATAAAATAATAACACATACGT
AAAGATTTTTACAAGACAAGATTGAAAACTTCTTAAAAGTACGTATAAAACATCATCTGTATTTATAATTGTTTAA
CATTTAACAAATAGCCCTACTAATTGTGTTATGGAAATGTATAAATTGTCATTTAACATAACTTGTTTGAGTTTAT
CATTATTTGTTTTTGTTTGTTTTTACACAGTTGGCAAGCATGTTGGCAAGGCGGCCCTTACGTAAGGACTTCTACC
ATCATTACTGTATAATTTTGATAGTATTATCACCAGTACTGTTATTGACAACTTCTCTTGTCCTGCTGACTCTCTC
CATCCGACTCATCCATAGTGCTTACCTTGGCGACAAGCAAGAACTCGACAAGCGCGCAGTCGATGA
//
NRC-08 Winter Flounder WFX (SEQ ID NO: 88)
TAATAAAACTAATGTGTAAAGTCTTCCACTTTTTTTACTGTATTTACTTAAACAGAAAATTATTCTCACGATTCTG
GAGCTGCAGCCACTAAGTGTTGCTTCATGAAGTGAATACACAATTGTTCTAACAACCACTCACCCAATTAACCAGA
ATCTACAAAGTGAGGAAGTGAGAGGAGTCGTCCTGTGTTTTCAAATTTTTTGAATGATCTACCACTATGTGAGCTC
CTCCTGTTATAGCTCTAAATGTTACACAATGAATGTGAAGTCAGTTCTGTGTATATAAAGAGTTGCCTCTGTAGAG
CATACAACAGATTTCACCTTTGAATCTCACAAACCTCACTTTGTATTCGACAGGTAAGATCGATATTTTTCAAACT
CATTTAGACGAGACCAAGTATTTGGGAAATGTGCTCAGCTTGTCAATGTATAATGCAAATGTTAACAATCGTTTTG
TTCTTATGTTGTGTTTGTAGGATGAAGTTCGCTACTGCCTTCCTGATGTTGTCCATGGTCGTCCTCATGGCTGAAC
CTGGAGAGTGTCGTTCTACAGAGGACATCATCAAGTCTATCTCGGGTAGAGTCCAGGAATTAATTATTATCAATAA
CAATGAAATAACAACCAAAAGGCCTCTGATTAGCATGTTCCTTCAATGAAATGGTCGTTTTTTATCTATTTTGATT
CTCACATGCAACGACCTGCTGCGGCAACATTTGAAAATCAATCTTTTTTACACAAATTCAAAGTACATTGATTTAT
TCGATTTAATCTTAACATTAATCAGATTTGTTTTTGTTTAAATATATCGAATAACTGGATCTCTATGATAAAATAA
TTAAACATACATTCTTATTTTACCAATCAAGATTGAACACTTCTTAAAAGTACGTATAAAACATCATCTGTATGTA
TAATTGTTTGATTGTTAAGTAATATTTCCAATAATTGTGTAATGGAAATGTATTAATTGTCATTTAATATAATTTG
CTTGAATTTATCACCATGTGTTTTTTGTTTGTTTTTAAACAGGTGGAGGTTTTCTCAATGCGTAAGGACTTCTATC
ATCATTACTGTGTAATTTTTATAGTATTATCATCAGTACTGTTATTAACAGCTTCTCTTGTCTCACTGACTCTCTC
CATCAGAATGAACGCCGGTTACAATGAGCAGCAGGAGCTCAACAAGCGCTCAGATGATGATGACAGCCCCAGTCTT
ATTGTTTTTGACTGAAGAAGTCGCCCTGAAGGAGCCTTCAGATGATATATTATGCTTCTTGCTCTTCATTGAAATA
AATCAAAC
//
NRC-09 and NRC-10 Winter Flounder WFY and WFZ (alternative splice products
from the same pseudogene) (SEQ ID NO: 89)
GAGCTCGATCAAACCAGACAAAGTTGCCTTCCTTCACAACAATAGAGTGGAAGAGAAAACAGGAGAGGACTTGTAT
CCTCCTGATGCTGAGAAGAAGAAATAAGAAAGACTTGCAGCATTGATACTTTTACTTATACAGAAAACCTATAAAC
ATGACGGGAGCATAAGTTAAAGTCACAATACAGAAGAGAACCAGAAGCCAAACTGCAGCAAATTTACTGGTATTCA
TATGATACTGGAGCCAAAGCAACGCAGAGACTCAGCAGCAGTGAACCAAAGAGTTTAACTGTACTTGTGTCCAGGT
TGAATGAAAGTATTGAATAAAAAAAACCAAGACAGAACATGCATATTTTTTTGGAATGGAATATAAGTCAGGAGAA
TATGTGTTGTTGTGGTGGCAGGATCCATCACTCTGTCAAGTTAACACAAGAACTTTTAGAAACATAGATACGATCT
CAAGTAAACTTCCATTTACTATTTGACTTTTTTTAAATACTTACAAATTATATTTTAAAAAGCAACAATAAATCAG
AGATAACTTCATGGAGAAGTCTATATTCATATTTGTGAGCTGAACATTCATGCTGCCTGTTCTATCACATCTGAGT
GTGGAGGCCACTGACGTTTACTGACCTCAACGTCTACCGCTCTAATGCATTTGGAGTTAAAGGTAAGCATTTTGTT
ATTTGTCTTCACTGTATTGATACTAAATATACAGGGTTACAAATACAGTTAAAACAAGAGAGACGAGGTGTCGAAA
GCTTCAGCATCAATGTGCTGATCGCTGATAGCTGATCTTACCCGACACCGGTGACATGGCATCAAAATGACCACCT
CTTTTTTCTTCTCTTTTTTTTTGTAGGACGAAGTTCGCTGCCGCCTTCCTCGTGTTGTTCATGGTCATCGTCATGTT
TGAACCTGGAGAGTGTTTTTTTAGATTGCTTTTTCACGGGGTCCACCATGGTAGGGTCCCGGAAGTAATTTGATTA
TTACATGCCAAATATTTTAATGAAACATACCTTATGAGTAGTTGTATTATTTGGACAAGTAGAATCTCTATGATTT
CAGTAGTAATTAGAATAACAATCAAAAAGGCCTTTGATTAGCATGTTTCTTCAATGAAATGGACATTGAGGTTTAT
TTTGATTCTCACATGCTACAGCAACAATTGAAATCAAATTTTTCGCAGAAGAAACTTAATTAACATTGTTGTGCAA
TAGTGCTTAAAAAGTGTTACCATGGAATGGTGTGCGTTTAGGCACTCAATAAATTTGGTTATCAAAATTAAATTAA
AAAAATTAATATTTAAAATATTAATATTAAATCATAACTTTAATTGTTTAAAGTTCTCGCGGGGAACCACCCTTCT
TCTGAAGGTAAAGGATAGCCAATTTATTGATTAAGATCAGTCTCATTTAGATCTAGTTCAAATAGAAATCTCAATA
TTTTACCATCGAAGATTTTATAATGAACAGTGAAGGTTATGGAGTTCTAAACAGTGTAACAGTTGGCAAAGTTCAC
TATTGCAATATTAATGACAGACCATTTGTGAAAGAAGAACATTTATTATGAGCATAATAAAGTATGAAAGCACGAA
TTACTAAACAATCAAAGCTAACTAACAAGGACGTGTGTGGGTGTGTGTGTGAATGTAAATAAGGGGGGGGCTCAAA
CTGGTGGCCTACAAGAAGAGCCTTAAGATAGCAACCACAAGGGCTGTACCATAAATGTTGTAGTAAAAAGAGTTAT
TAAAATGAGTTAGAATAACTAATGACTAATTAGTAGACAAACTAGTAGACAAACTAAACAACTAACAATAACAAGG
AAGTGTGTGTGAGTGTGTTTGTGTGTAAATGTTAATTAGGGGCTCTCAAACTGGTGTCTTACCAGAAGAGTAAGAT
AACAATTCCCCCCCTTCTTCTGAGGTTGTTTTACGACTGTTGCTTTATGGCCGTGAGGGAAGGTTTAACTCGGTGA
CATGCTATACGTGTCTGTGTAGATGTTAATCAGAGAATGCCAGAGTCAGAGAGACCTACGGAGGAAGTCTGTGAAG
GGCCTATCTAACATTAGCTTTCCTTTAACTTATAACACAATATCAGAAACACATATCAACCTTATAAACACACACA
GAATCAAATAAACAGTCTTGCTTAGCATGTATAATTATTAAGCCCAGATTATGTTACCAGTCCGAGGGAAAGAGTT
CAGTTGCAGTTCTGTGACGTCTCCTGGCTTTGTGGTCGTAGAGTTCTGCATTCGCGATTCTGTCGAGCCGTGTGCT
CAGATGCAGGTTGAAGTTCTCCTGCAGGACATCGCGTCGCTGCGAGGATTTTGTAGAGCTTGAAGGGCGAGGAGAT

TTCCTTGAGTGGTGAGCTGGAAGCTGGACCTCTGACCTCTGGTTGTTGGTTGGAAGAGAAGAAAGCTGGAGCGGCG
TGGTTTCTCCCTCTAGCCGATGCAGGAGGAGAAGCCGGCAGCCCCACTCCTTGAAGAGTTGTGGAGAGAGATGGGA
GCAAAGAGCTAGATTTTGGGGAGACCTCTCCTTATATTGGCCCCGATGACCTCACAGGCCTTGGAACGGAGTGACC
AATAGGAGTTGACCCTGGTAATTCTTGACACCTTTGTGGGACATTGTCAAGACCCCAGGACATGCAGCATCCTGTT
ACAATCTGGGAGACGGAGTTCCTTGACTGTCTCAGAACAATGAGAACCTGTGGCATCTTGGGGGATTGAGTCCACT
CGAGCACATGCGGCATGTTTGTTCCAAGTTTGACTGAAAGGAGGCCTGTGGTTTGCACAAAAACCATGTCCCAACA
ACATTTTCTAGGCGATTTAATCTTTACATAAATTGGATTTGTTTTAAAAAATATATAGAATAACTCGATCTTTCTG
CGTAAATAATAAAAAATAAATTCAAATTTGACCAGTCAAGATTGAACACTAATGAAAAGTACCTATAAAACATAAT
CTGTATGTATAGTTGTTTGACTGTTAAATAGTAGTCCTAACAATTGTGTAATGGAAATGTATTCATTGTCTTTTAA
TACTATTTGCTTATCATAATGTGTTTGTTTGTTTTTTAGCAGGTGGAGGTTATCTCAATGCGTAAGGACTTCTACC
ATCATTACTGTGTAATTGTATTAGTTTTATCATCAGTACTGTTATTGACAACGTCTCTTGTCTTGCTGACTTGACT
CTCTTCATCAGATTAAACCCAGGGCCGGTTACAATGAGCAGCAGGAGCTCGACAAGCGCGCAGTCGATGACAACCT
CAGTGCTATTGTTTTTTACTGAAGAAGTCGACCTGAAGAATCTTTTGAAATGATATGAAATGTTTGCCTTTCAATG
AAATAAATCAAACATGACTGGATATTTGTTCTTTTGCATTGATGTATTGTTGAGTGACAGTTGAATAATTTTGGAA
AACTTATAACAGATCTCAATTTTAGGATGTCAAATCATTTCTCTGTGTCTTATTCAAATATGAGATTTAACAATGA
CAAT
//
NRC-11 American Plaice AP1 (SEQ ID NO: 90)
GCCCACTTTGTATTCGCAAGGTAAGATCAATATTTTTCAAATTCATTTAGACGAGACCAACCGTTTGCGAAATGTG
CTCAGCTTGTTATTGTATAATAACAAAGTTAACGATCTTTATTTTTCTGTTTTTTTTGTAGAATGAAGTTCACTGCC
ACCTTCCTGATGTTGTTCATCTTCGTCCTCATGGTTGAACCTGGAGAGTGTGGATGGAAAAGTGTGTTTCGTAAGG
CTAAGAAAGGTAGAGTCACGGAATTAATTAGCTTTTTACATTGCAAATAGATTTTTTATAACAGCTGGAAAATCAC
AAAAATAAATAGTCGATATATTTGGCCAATTAGAATCACTTTAATTTCAATAATAATCTAAATAACAACCTAAAAG
GCCTTTGATTAGCATGTTTCTTCAATGAAATGGACATTGAGGTTTATTTTGATTCTCACATGCACCGACCTGTGCG
GCAACCATTGAATTCAGATTTGTCCCAGAAGAATTCAAAGTACATTTTTCCAGGCGATTAAATCTTTCCATTACTC
AGATTCAAAAATAAATAAATGGAATAATTGAAGCACTATGATAAAATAATTACACATTCACTCTGACTTTACAAGT
CAAGATTGAACACTATTAAAAAGTGTGTATAAAACAACATCTGTATGCATAATTGTTTAACTGTTAATAGTCCTAA
TAATTGTTTTATGGAAATGTATTAATTTACATTTAATATTATTTGCTTGAGTTTACCATCATGTGTTTTTGTTTGT
TTTTACACAGTTGGCAAGACTGTTGGCGGCTTGGCCCTTGAGTAAGGACTTCTACCATCATTACTGTATAATTTTG
ATAGTATTATCACCAGTACTGTTATTAACTACTTCTCTTGTCTGCTGACTCTCTCCATCCGACTCATCTGCAGTCA
TTACCTTGGCGAGCAGCAGGAGCTTGACAGCGCGCAGTCGATGAGGACCCCAGTGCTATTGTCTTTGACTGAAGAA
GTCGCCTTGAAGGAG
//
NRC-12 American Plaice AP2 (SEQ ID NO: 91)
ACTTTGTATTCGCAAGGTAAGATCAATATTTTTCAAATTCATTTAGACGAGACCAACCGTTGGCGAAATGTGCTCA
ACTTGTTATTGTATAATAACAAAGTTAACGATCTTTATTTTTCTGTTTTTTTTGTAGAATGAAGTTCACTGCCACCT
TCCTGATGTTGTTCATCTTCGTCCTCATGGTTGAACCTGGAGAGTGTGGATGGAAAAAATGGTTTAATAGGGCTAA
GAAAGGTAGAGTCACGGAATTAATTAGCTTTTTACATTGCAAATAGATTTTTTATAACAGCTGGAAAATCACAAAA
ATAAATAGTCGATATATTTGGCCAATTAGAATCACTTTAATTTCAATAATCTAAATAACAACCTAAAAGGCCTTTG
ATTAGCATGTTTCTTCAATGAAATGGACATTGAGGTTTATTTTGATTCTCACATGCACCGACCTGTGCGGCAACCA
TTGAATTCAGATTTGTCCCAGAAGAATTCAAAGTACATTTTTCCAGGCGATTAAATCTTTCCATTACTCAGATTCA
AAAATAAATAAATAGAATAATTGAAGCACTATGATAAAATAATTACACATTCACTCTGATTTTACAAGTCAAGATT
GAACACTATTAAAAACTGTGTATAGAACATCATCTGTATGTGTAATTGTTTAACTGTTAATAGTCCTAATAATTGT
TTTATGGAAATGTATTAATTTACATTTAATATTATTTGCTTGAGTTTACCATCATGTGGTTTTGTTTGTTTTTACA
CAGTTGGCAAGACTGTTGGCGGCTTGGCCGTTGAGTAAGGACTTCTACCATCATTACTGTATAATTTTGATAGTAT
TATCACCAGTACTGTTATTAACTACTTCTCTTGTCTCGCTGACTCTCTCCATCCGACTCCTCTGCAGTCATTACCT
TGGCAAGCAGCCGGAGCTCGACAAGCGCGCAGTCGATGAGGACCCCAGTGCTATTGTCTTTGACTGAAGAAGTCGC
CTTGAAGGAGCCTTCAGAA
//
NRC-13 American Plaice AP3 (SEQ ID NO: 92)
TTGCCCACTTTGTATTCGCAAGGTAAGATCAATATTTTTCAAATTCATTTAGACGAGACCAACCATTTGGGAAATG
TGCTCAGCTTGTTACTGTATAATGCAAAAGTTAAGTATCTTTATTTTTCTGTTTTTTTTTGTAGAATGAAGTTCAC
TGCCAACTTCCTCATGTTGTTCATCTTCGTCCTCATGTTTGAACCTGGAGAGTGTGGTTGGCGAACATTGCTTAAA
AAAGCTGGTCACGGAATTAATACGCTTTTTACATTGCAAATAGATTTTTTATAACAGCTGGAAAATGACAAAAATA
AATAGTCGATATATTTGGCCAATTAGAATTATTTTGATTTCAATAATAATCTAAATAACAACCTAAAAGGTCTTTG
ATTAGCATGTTTCTTCAATGAAATGGACATTGAGGTTTATTTTGATTCTCACATGACCGACCTGCTGCGGCAACAA
TTGAATTCAGATTTGTCCCAGAAGAATTCAAAGTAAATTTTCCAGGGGATTAAATCTTTCCATTACTCGGATTTAA
AAAAAAAAAAAATAGAATAACTGAATTGCCATGAAAAAATAATTACACATACTGTCTGATTTTACAAGTCAAGATT
GAACACTACTTAAAAGTATGTATAAAACATCATCTGTATGTATAATTGTTTAACTGTTAACAAATAGTCCAAATAA
TTGTGTTATGGAAATGTATTAATTGTCATTAAATATAATTTGCTTGAGTTTATCATCATGTGTTTTTTTTTTTTTT
TTACACAGAGGTTAAGACTGTTGGCAAGTTGGCCCTTAAGTAAGGACTTCTACCATCATTACTGTATAATTTTGAT
AGTATTATCACCAGTACTGTAGTACTGACAACTTCTCTCTCCACCCAACTCATCCGCAGACATTACCTTGGCAAGC
AGCCGGAGCTCGACAAGCGCGCAATTGATGACGACCCCAGTATTATTGTTTTTGACTGAAGAAGTCGCCTTGAAGG
AGCCTTCAGAA

//
NRC-14 Witch Flounder GcSc4C5 (SEQ ID NO: 93)
ATGAAGTTCACTGCCACCTTCCTCATGATGTTCATGGTCGTCCTCATGGCTGAACCCGGAGAGGCTGGTTGGGGAA
GTATTTTCAAACATATTTTCAAAGCTGGAAAGTTCATCCATGGTGCGATCCAGGCACACAATGACGGCGAGGAGCA
GGATCTCGACAAGCGCGCAGTCGATGA
//
NRC-15 Witch Flounder GcSc4B7 (SEQ ID NO: 94)
ATGAAGTTCACTGCCACCTTCCTGGTGTTGTTCATGGTCGTCCTCATGGCTGAACCTGGAGAGGGTTTTTGGGGAA
AGCTTTTGAAATTGGGCATGCATGGAATCGGGCTGCTCCATCAGCATTTGGGTGCTGACGAGCAGCAGGAGCTCGA
CGAGCGCTCAGAGGAGGACGAGCCCAATGTTATTGTTTTTGAATGAAGAAGTCGCATTGAAGGAGCCTTCAG
//
NRC-16 and NRC-17 Witch Flounder GC3.8 (SEQ ID NO: 95)
ATGAAGTTCACTGCCACCTTCCTGGTGTTGTTCATGGTCGTCCTCATGGCTGGATCCGGAGAGTGTGGTTGGAAAA
AGTGGCTCCGTAAAGGTAGAGTCATGGATTTAATTTGCTTTTTACATTGCAAATACTTTAATATAACATAGTTGGA
AAACCACAAAAATAAGTAGTCGATATATTTGGCCATATAGAATCACTTTGATTTCAATAATAATCAAAACAACAAT
CAAAAAGCCCATTGATTAGCATGTCCCTTCACTAAAATGGACATTGTAATTTATTTTGATTCTCACAGGCACCAAC
CTGCTGCGGCAACAATTGAAATCAAATTTGTCTCAGAAGAATTCAAAGTACATTGTTCTAGGCGATTTAATCTTTC
CATTCATCGGATCTGTTTTTAAAAATATAGAATAACTGGATCTCTATGTTAAAATAATAAAACACACATTCTGATT
TTACCTGTCAAGATTGAACACGACTTAAAAGTATGTATAAAACATCATCTGTATGTATAATTGTTTAACTGTCAAC
TAATAGTCCAAATAATTGTGTTATGGAAATGTATTCATTGTCATATAATATCATTTGCTTGAATTTATCACCATGT
GTTTTTGTTTGTTTTTACACAGGTGCCAAGCACCTTGGCCAGGCGGCCATTAAGTAAGGACTTCTACCATCATTAC
TGTGTAATTTTAACAGTATTATCATCAGTACTGTTATTGACAACTACTCTTGTCTCTGTTACTCTCTCCAGGGGTT
TGGCCTCTTGCGAAGAGCAGCAGGAGCTCGACAAGCGCTCAATGGATGACGAGCCCAGTGCTATTGTTTTTGACTG
AAGAAGTCGCCTTGAAGGAGCCTTCA
//
NRC-18 Witch Flounder GC3.2 (SEQ ID NO: 96)
ATGAAGTTCACTGCCACCTTCCTGGTGTTGTTCATGGTCGTCCTCATGGCTGGATCCGGAGAGTGTGGTTGGAAAA
AGTGGTTCACTAAAGGTAGAGTCATGGATTTAATTTGCTTTTTACATTGCAAATACTTTAATATAACATAGCTGGA
AAATCACAAAAATAAGTAGTCGATATATTTGGCCATATAGAATCACTTTGATTTCAATAATAATCAAAACAATAAT
CAAAAAGCCTATTGATTAGCATGTTCCTTCACTAAAATGGACATTGTAATTTATTTTGATTCTCACAGGCACCAAC
CTGCTGTGGCAACAATTGAAATCAAATTTGTCTCAGAAGAATTCAAAGTACATTGTTCTAGGCGATTTAATCTTTC
CATTCATCGGATTTGTTTTCAAAAATATAGAATAACTGGATCTCTATGTTAAAATAATAAAACACATTCTGATTTT
ATCTGTCAAGATTGAACACGACTTAAAAGTATGAATAAAACATCATCTGTATGTATAATTTTTTAACTGTCAACTA
ATAGTCCAAATAATTGTGTTATGGAAATGTATTCATTGTCATATAATATCATTTGCTTGAATTTATCACCATGTGT
CTTTGTTTGTTTTTTACACAGGTGAAAGGTTATCCCAGAGGTAAGGACTTCTACCATCATTACTGTATAATTTTAAT
AGTATTATCATCAGTACTGTTATTGATAACTTCTCTTGTCTCGCTGACTCTCTCCATCAGGCATTTCGCTGACGTC
GAGCAGCAGGAGCTCGACAAGCGCTCAGTGGATGACGAGCCCAGTTCTATTGCTTTTGACTGAAGAAGTCGCCTTG
AAGGAGCCTTCAG
//
NRC-19 Halibut HB26 (SEQ ID NO: 97)
TTATGAAGTTCACTGCCACCTTCCTGGTGTTGTTCATGGTCGTCCTCATGGCTGAGCCTGGAGAGTGTTTTTTGGG
ATTGCTTTTTCACGGGGTCCACCATGGTAGGGTCACGGAAGTAATTCGATTTTTACATGGCAAATATTTTAAGATA
ACACACCATATGAGTAGTCGATATATTTGACCAATTAGAATCACTTTAATTTCAATAATAATCACAATAACAATCT
CTAGGCCATTTAATCTTTCCATTAATCGGATTTGTTTTTTTAAATATAGAATAACTGGATCTCTATGTTAAAATAA
TAAAACATACATTCTGATTTTACCAGTCAAGATTGTACGCTACTTAAAAGTATGTATAAAACATCATCTGTATGTA
TAATTGTTTAACTGTTAACTAATAGTCCAAATAATTGTGTAATGGAAATGTATTAATTGTCATTTAATATCATTTG
CTTGAATTTATCACCATGTGTTTTTGTTTGTTTTTACACAGTTGGAAAGTGGATCCATGGGTAAGGACTTCTACCA
TCATTACTGTGTATTTTTAATAGTATTATCATCAGTACTGTTATTGATATTTTCTCTTGTCTCGCTGACTCTCTCC
ATCAGACTCATCCATGGGCATCACGGTTACGACGAGCAGCAGGAGCTCGACAAGCGCGCAGTCGATGAAA
//
NRC-20 Halibut HB18 (SEQ ID NO: 98)
TTATGAAGTTCACTGCCACCTTCCTGGTGTTGTTCATGGTCGTCCTCATGGCTGAACCTGGAGAGGGTTTTTTGGG
AATTCTTTTTCACGGGGTCCACCATGGTAGAGTCACGGAATTAATTCGATTTTTACATGGCAAATATTTTAAGATA
ACACACCATATGAGTAGTCGATATATTTGACCAATTAGAATCACTTTAATTTCAATAATAATCACAATAACAATCT
CTAGGCCATTTAATCTTTCCATTAATCGGATTTGTTTTTTTAAATATAGAATAACTGGATCTCTATGTTAAAATAA
TAAAACATACATTCTGATTTTACCAGTCAAGATTGAACACTACTTAAAAGTATGTATAAAACATCATCTGTATGTA
TAATTGTTTAACTGTTAACAATAGTCCAAATAATTGTGTTATGGAAATGTATTAATTGTCATTTAATATCATTTGC
TTGAATTTATCACCATGAGTTTTTTGTTTGTTTTTACACAGGTAGAAAGAAGGCCTTGCAGTAAGGACTTCTACCA
TCATTACTTTGTAATTTTTATAGTATTATCATCAGTACTGTTATTGACAACTTCTCTTGTCTCGCTGACTCTCTCC
ATCAGGATGAACTCAGAGCGTCGCAGTTACGACGAGCGGCAGCAGCAGCAGGAGCTCGACAAGCGCGCAGTCG
ATGAAA
//
NRC-101 Yellowtail Flounder YT1 (SEQ ID NO: 99)
GCCCACTTTGTATTCGCAAGGTAAGATCGATATTTTTCAAACTCATTTAGACGAGACCAAGCATTTGTTGAAATGT

GATAAGCTTCTAACTTTATAATGCAAATGTTAACAATCTTTTTGTTCTGTTGTTTTTGTAGGATGAAGTTGGCTGC
CGCCTTCCTGGTGCTGTTCCTGGTCGTCCTCATGGCTGAACCTGGAGAGGGTTTCTTGGGATTTCTTTTTCACGGT
ATCCACCATGGTAAAGTCACTCATTTAATACATTTTTACATGGCAAATATTTGAATATAACATACTATATGAGTTG
TCAATATATGTGGCCAAGTAGAAGCACTTTGATTTCAATAATAATCAAAATAACAATCACTAAGCCATTTAATAAT
TGAATTAATTACATTTGTTTTAAAAAAATATAGAATAACTGGATCTTTATGCTAAAATAATTAAACCTAAATTCAG
ATTTTACCACTCAAGATTGAACACTACTTAAAAGTATGTAAAAAAAACATCATCTGTATGTATAATTAAATACTAG
TCCAGTTAATTGTTTTATGGAAATGTGTTAATTGACATATATCATTTGCTTGAACTTATAATGTGCTTTGTTTGTT
TTTACACAGGTATCAGGGCGATCCATCAGTAAGGACTTCTACCATCATGACTGTGTATTTTTAATAGTATTATCAT
CAGTACTTTTATTAACAACTTCTCTTGTCTCGCTGACTCTCTCCATCAGTCTCATCCATGGTCAAAGATACGACGA
GCAGCAGGAGCTTGACAAGCGCTCAGTCGATGACAACCCCGGTGCTATTGTTTTTGACTGAAGACGTCGCCTTGAA
GGAGCCTTCAG
//
NRC-102 Yellowtail Flounder YT3 (SEQ ID NO: 100)
ATGAAGTTCACTGCCACCTTCCTGGTGTTGTCCATGGTCGTCCTCATGGCTGAACCTGGAGAGGGTTTCTTTGGAG
CCCTTATCAAAGGGGCCATCCATGGTGGCAAGTTGCTCCATAAACTCATCAAAAAAAAACATGAACATCACGGTTA
TGGCAAGCATTGGGGGCTTGACAAGCGCGCAGTCGATGA
//
NRC-103 Winter Flounder WF-YT (SEQ ID NO: 101)
TTGAAAGTGAGGAAGTGAGAGGAGGACTAGGTCCTGTGTTTTCAGTCGTTGAATTATCTAACACTATCTGAGCCCC
TCCTGCAATAACTCTAAATGTTACACAGTGACTAGGAAGTCAGTCCTGTGTATATAAAGAGTTGCATCTGTTGTTA
TCAGTAGACAACAGATTACACCTTTGAATCTCACAAAGCTCATTTTGTATTCGACAGGTAAGATCGATATGTTTCA
AACTCATTTAGATGAGACCAAGCATTTGGGAAATGTGCTCAGCTTCTAACTGTATGATGCAAATGTTAACAATCTT
TTTGTTCTGTTGTTTTGTAGGATGAAGTTGGCTGCCGCCTTCCTGGTGCTGTTCCTGGTCGTCCTCATGGCTGAAC
CTGGAGAGAGTTTTTTGGGATTTCTTTTTCATGGTATCCGCCATGGTAGGGTCACTGAATTGATACATTTTTACAT
GGCAAATATTTGAATGTAACATACTATATGAGTTGTCAATATATGTGGCCAAGTAGAAGCACTTTGATTTCAGTAA
TAATCAAAATAACAATCACTAGGCCATTTAATAATTGCATTAATTACACTTGTTTTTATATAGAATATAGAATAAC
TGGATCTTTATGCTAAAATTAATAAACATGAATTCAGATTTTAAGATTTTTCAAGATTGAAAACTACTTAAAAGTA
TGTAAAAAAACATCATCTGTATGTATAATTAAATACTTGTCCAGATAATTGTGTTGTGGAAATGTGTTAATTGACA
TATATCATTTGCTTGAATTTATCATTATCTGCTTTGTTTGTTTTTACACAGGTATCAAGGCGATCCATGGGTAAGG
ACTTCTACCTTCATGACTGTGTATTTTTAATAGTATTATATTCAGTACTGTTATTGAAAACTTCTCTTGTCTCGCT
GACTCTCTCCATCAGAATGATCCATGGTAACAGTTTAGACGAGATGCAGGAGCTCGACAAGCGCTCATTCGATGAC
AACCCCAACGCAATTGTTTTTGACTGAAGAAGTCGCCCTGAAGGAGCCTTCAGATGATATATAATGCTTCTTGCTT
TTCAATGAAATAAATTGAATAATTACCCGCAACAGC
//
NRC-104 Winter Flounder WF1-like (SEQ ID NO: 102)
TACTTTTATCTACCACTATGTGAGCTCCTCCTGTTATAACTCTAAATGTTACACAATGAAGATGAGGTCAATTCTG
TGTATATAAAGAGTTGCCTCTGTATAGTAGACAACATATTTCACCTTTGAATCCCACAAAGCTCACTTTGTACTCA
ACAGGTAAGATCGATATTTAAAAACTAATTTAGACGAAACCAAGCATTTTGGGGAATTTGCTCAACTTCTAAATGT
ATGATACAAATGTTAACAATCTTTTATTTCTGTTGTTGTTTTTTGTAGGATGAAGTTCACTGCCACCCTCCTCCTG
TTGTTCATCTTCGTCCTCATGGTTGATCTCGGAGAGGGTCGTCGTAAGAAAAAGGGGTCGAAGAGAAAGGGGTCCA
AGGGAAAGGGGTCCAAGGGAAAGGGCAGGTGGTTGGACAGGATTGGTAAAGGTAGAGTCACGGAATTAATTTGCTT
TTTACATTGCAAATATTTTTCATATAACATTGCTGGAAAATCACAAAAATAAGTAGTCAATATATTTGGCCAAATA
GAATCACTTTGATTTCAATAATAATCAAAATAACAACCTAAAAGGCCTTTGATTAGCATGTTCCTTCAATGAAATG
GACATTGTAATTTACTTTGATTCTCACATGCTACGACCTGCTGCAGCAACATTTGAAAATAAATTTGTCCCAGAAG
ATTTTAAAGTACATTGTTATAGGCGATTTATCTTTCTATTACTCAGATATTTGTTCAAACCAATAGAATAACTGGA
TCTCTATGCTAAAATAATAAAACACACATTCAGATGTTACCAGTCAAGATTGAACGCTGTTTAAAAGTAAGTATGA
AACATCCTCTGTATGTATAATTGTTTAACTGGTAACTTATAGTCCTAATAATTGCGTTATGGAAATGTATTAATTG
TCATTTAATATAATTTGCTGGAATTTATCACTGTGTGTTTTTGTTTGTTTTTACACAGCTGGCGGGATAATTATCG
GGGGGGCCCTTGAGTAAGGACTTCTACCATCATTACTGTGTAATATTTATAGTTATGATCAGTACAGTTATTAACA
ACTTCTCTTGTCTCGCTGAACTTCTCCATCAGTCACCTCGGGCAGGGGCAGGTGCAGGGGCCGGATTACGACTACC
AGGAGGGGGAGGAGCTCAACAAGCGCTCAGACGATGATGACAGCCCCAGTCTTATTTTTTTTGACTGAAGAAGTCG
CCCTGAAGGAGCCTTCAGATGATATATAATGCTTCTGGCTTTTCATTGAAATAAATAATACGTTTACCTGCAACAG
CAACCATG
//
NRC-105 Halibut Hb29 (SEQ ID NO: 103)
TTATGAAGTTCACTGCCACCTTCCTGGTGTTGTTCATGGTCGTCCTCATGGCTGAACCTGGAGAGGGTTTGGGAAA
TTGGATGGGGCCCCATATCAGCGGTAGAGTCACGGAATTAATTTGCTTTTTCCATTGCAAATATTTTAATATTGCA
TAGCTGGAAAATCACGAAATAAGTAGTCGATATATTTGGCCAAATAGAATCACTTTGATTTCAATAATAATCAAAA
TAACAATCAAAAAGGCCTTTGATTAGCATGTTCCTTCAATAAAATGGACATTGAAGTTTATTTTGATGCTCACATG
CACCGACCTGCTGCGGCAACAATTGAAATCAAATTTGTCTCAGAATTTAAAGTACATTTTTCTAGGTGATTTAATC
TTTCCATTAACTTGATTTGTTTTTATAAATATAGAATAACTGGATCTTTATGCCAAAATAATAAAACACACATTCT
GATTTTACCAGTCAAGATTGAACACTACTTAAAAGTAATATAAAACATCATCTGTATGTATAATTGTTTAACTGTT
AACAAAAGTCCAAATAATTGTGTTATGGAAATGTATTAATTGTCATTTAATATCATTTGCTTGAATTCATCACCAT
GTGTTTTTTGTTTGTTTTTACACAGGTGAAAAGAAGGCCTTGCAGTAAGGACTTCTACCATCATTACTTTGTAATT

TTTATAGTATTATCATCAGTACTGTTATTGACAACTTCTCTTGTCTCGCTGACTCTCTCCATCAGGATGAACTCAG
AGCGTCGCAGTTACGACGAGCGGCAGCAGCAGCAGGAGCTCGACAAGCGCGCAGTCGATGA
//
NRC-106 Halibut HbSc1A13 (SEQ ID NO: 104)
ATGAAGTTCACTGCCACCTTCCTGGTGTTGTTCATGGTCGTCCTCATGGCTGAACCTGGAGAGGGTTTGGGAAATT
GGATCGTGCGCCCTATCGGAGGTGAAAAGAAGGCCTTGCAGATGAACTCAGAGCGTCGCAGTTACGACGAGCGGCA
GCAGCAGCAGCAGGAGCTCGACAAGCGCGCAGTCGATGAAA
//
NRC-107 Halibut HbSc1A24 (SEQ ID NO: 105)
ATGAAGTTCACTGCCACCTTCCTGGTGTTGTTCATGGTCGTCCTCATAGCTGAACCTGGAGAGAGTCTTTTTGGAA
AGTTCCTCAAGAAAGTTGTCCATGCTGGCACGTCAATTGGCGAGACAGCCTTGCATGTCGCCGCAGAGCATCACGG
GCTTCATGCGCATCACGGGTGTCACGGGCGTCACGGGGGTCACAGGCGTCACGGGGGTCACAGGCGTCACGGGCGT
CGCGGTTACGACGAGCAGCAGCAGGAGGAGCTCGACAAGCGCGCATTCGATGA
//
NRC-108 Halibut HbSc1B34 (SEQ ID NO: 106)
TATGAAGTTCACTGCCACCTTCCTGGTGTTGTTCATGGTCGTCCTCATGGCTGAACCTGGAGAGGGTTTGGGAAAT
TGGATGGGGCCCCATATCAGCGGTAGAAAGAAGGCCTTGCACATGAACTCAGAGCGTCGCAGTTACGACGAGCGGC
AGCAGCAGCAGCAGGAGCTCGACAAGCGCGCAGTCGATGAAA
//
NRC-109 Halibut Hb17 (SEQ ID NO: 107)
ATGAAGTTCACTGCCACCTTCCTGGTGTTGTTCATGGTCGTCCTCATGGCTGAACCTGGAGAGTGTTTTTTGGGAT
TGCTTTTTCACGGGGTCCACCATGGTAGGGTCACGGAAGTAATTCGATTTTTACATGGCAAATATTTTAAGATAAC
ACACCATATGAGTAGTCGATATATTTGGCCAATTAGAATCACTTTGATTTCAATAATAATCAAAATAACAATCTCT
AGGCGATTTAATATTTGCATTAATTGGATTTGTTTTTAAAAATATAGAATAACTGGATCTTTATGGTAAAATAATT
AAACATACATTCTGATTTTACCAGTCAAGATTGAACACTACTTAGAAGTATGTATAAAACATCATCTGTATGTATA
ATTGTTTAACTGTTAACGAATAGTCCAAATAATTGTGTTATGGAAATGTATTAATTGTCATTTAATATCATTTGCT
TGAATTTATCACCATGTGTTTTTGTTTGTTTTTACACAGTTGGAAAGTTGATCCATGGGTAAGGACTTCTACCATC
ATTACTGTGTATTTTTAATAGTATTATCATCAGTACTATTATTGACAACTTCTCTTGTCTCGCTGACTCTCTCCAT
CAGACTCATCCATGGCGGTTACGACGAGCAGCAGGAGCTCGACAAGCGCGCAGTCGATGAA
//
NRC-110 Witch Flounder GC1.2 (SEQ ID NO: 108)
GCCCACTTTGTATTCGCAAGGTAAGAGCGATATATTTCAAATTCATTCGGATGAGACCAAGCATTTGGGAAATGTG
CTCAGCTTGTTACTGTTTAATGCAAATGTTAACAATATCCTTTTTCTGTTGTTTTTGTAGAATGAAGTTCGCTGCC
GCCTTCCTCATGATGTTCATGGTCGTCCTCATGGCTGAACCCGGAGAGGCTCGTTGGGGAACGTTCTTCAAACATA
TTTTCAAAGGTAGAGTCACAGAATTAATTTGCTTTTTACATTGCAAATATTTTCATATAACATAGCTGGAAAATCA
CAAAAATAAGGGCTTGATATATTTGGCAAAGTAGAATCCCTTTGATTTCAATAATAATCAAAATAAAAATCAGAAA
GGCCTTTGATTAGCATGTTCCTTCAATAAAATGGACATTGTAGTTTATTTTGATTCTCAAATGCACCAACCTGCTG
CGGCAACAATTGAAATCAAATTTGTCTCCGAAACATTTAAAGTACATTTTTCGAGGCAATTTAATCTTTCCTTTGA
TCGAATTCGTTTTTAAAAATATAGAATAACTGGATCTTTATGCTAAAATAATAAATCATACATTCTGATTTTACCA
GTCAAGATTGAACGCTACTTAAAAGTATGTATAAAACATCATCTGTATGTATAATTGTTTAACTTTTAACTAATAG
TCCTAATAATTGTGTTATGGAAATGTATTCATTGTCATTTAATATCATTTGCTTGAATTTATCACCATGTGTTTTT
GTTTGTTTTTACACAGCTGGAAGGTTCATCCATGGGTAAGGACTTCTACCATCATTACTGTGTATTTTTAATAGTA
TTATCATCAGTACTGTTATTGATAACTTCTCTTGTCTCGCTGACTCTCTCCATCAGTGCGATCCAGGCACACAATG
ACGGCGAGCAGCAGGATCTCGACAAGCGCTCAGTGGATGATGAGCCCAGTGTTATTGTTTTTGAATGAAGAAGTCG
CCTTGAAGGAGCCTTCAG
//
NRC-111 Witch Flounder GC1.3 (SEQ ID NO: 109)
GCCCACTTTGTATTCGCAAGGTAAGAGCAATATATTTCAAATTCATTTAGACGAGACCAAGCATTTGGGATCTGTG
CTCAACTTGTAACTGTATAATGCAAATGTTAACAATATTCTTTTTCTGTTGTTTTTGTAGAATGAAGTTCGCTGCC
GCCTTCCTCATGATGTTCATGGTCGTCCTCATGGCTGAACCCGGAGAGGGTGCTTGGATACCTGCCTTGAATAGGA
TCTATCATGGTAGAGTCACAGAGTTAATTTGCTTTTTACATTGCAAATATTTTAATATAACATGGCTGGAAAATCA
CAAAAATGAGTACTCGATATATTTGGCAAAGTAGAATCCCTTTGATTTCAATAATAATCAAAAACACAATCAAAAA
GGCCATTGATTAGCATGTTCCTTCAATGAAATGGACATTGTAGTTTATTTTGATTCTGACATGCACCAACTTGCTG
CGGCAACAATTGAATTCAAATTTGTCTCAGAAAAATTTAAAGTACATTTTTCTTTCCATTAGTCGGATTTGTTTTA
AAAAATACAGAATAACTGGATCTTTATGCTAAAATAATAAATCATACATTCTGATTTTACCAGTCAAGATTGAACG
CTACTTAAAAGTATGTATAAAACATCATCTGTATTGATAATTGTTTAACTTTTAACTAATAGTCCTAATAATTGTG
TTATGGAAATGTATTCATTGTCATTTAATATCATTTGCTTGAATTTATCACCATGTGTTTTTGTTTGTTTTTACAC
AGCTCTACTGAGGATCAATCGGTAAGGACTTCTACCATCATTACTGTGTAATTTTAATAGTATTATCATCAGTACT
GTTATTGATAACTTCTCTTGTCTTGCTGGCTCTCTCCATCAGCCAAATGGTGTATTATCGTCGGCACTGGCACGGT
GACGTCGAGCAGCAGGCTCTCGACAAGCGCTCAGTGGAGGACCAGCCCAGTTCTATTGCTTCTGCCTGAAGAAGTC
GCCTTGAAGGAGCCTTCAG
//
NRC-112 Witch Flounder GC1.4 (SEQ ID NO: 110)
GCCCACTTTGTATTCGCAAGGTAAGAGCAATATATTTCAAATTCATTTAGACGAGACCAAGCATTTGGGATCTGTG

CTCAACTTGTAACTGTATAATGCAAATGTTAACAATATTCTTCTTCTGTTGTTTTTGTAGAATGAAGTTCGCTGCC
GCCTTCCTCATGATGTTCATGGTCGTCCTCATGGCTGAACCCGGAGAGGGTGCTTGGATGCCTGCCTTGAATAGGA
TCTATCATGGTAGAGTCACAGAGTTAATTTGCTTTTTACATTGCAAATATTTTAATATAACATGGCTGGAAAATCA
CAAAAATGAGTACTCGATATATTTGGCAAAGTAGAATCCCTTTGATTTCAATAATAATCAAAAACACAATCAAAAA
GGCCATTGATTAGCATGTTCCTTCAATGAAATGGACATTGTAGTTTATTTTGATTCTGACATGCACCAACTTGCTG
CGGCAACAATTGAATTCAAATTTGTCTCAGAAAAATTTAAAGTACATTTTTCTTTCCATTAATCGGATTTGTTTTA
AAAAATACAGAATAACTGGATCTTTATGCTAAAATAATAAATCATACATTCTGATTTTACCAGTCAAGATTGAACG
CTACTTAAAAGTATGTATAAAACATCATCTGTATTGATAATTGTTTAACTTTTAACTAATAGTCCTAATAATTGTG
TTATGGAAATGTATTCATTGTCATTTAATATCATTTGCTTGAATTTATCACCATGTGTTTTTGTTTGTTTTTACAC
AGCTCTACTGAGGATCAATCGGTAAGGACTTCTACCATCATTACTGTGTAATTTTAATAGTATTATCATCAGTACT
GTTATTGATAACTTCTCTTGTCTTGCTGACTCTCTCCATCAGCCAAATGGTGTATTATCGTAGGCACTGGCACGGT
GACGTCGAGCAGCAGGCTCTCGACAAGCGCTCAGTGGAGGACCAGCCCAGTTCTATTGCTTCTGCCTGAAGAAGTC
GCCTTGAAGGAGCCTTCAG
//
NRC-113 Witch Flounder GcSc4B35 (SEQ ID NO: 111)
ATGAAGTTCACTGCCACCTTCCTGGTGTTGTTCATGGTCGTCCTCATGGCTGGATCCGGAGAGTGTGGTTGGAAAA
AGTGGTTCACTAAAGGTGCCAAGCACCTTGGCCAGGCGGCCATTAACGGTTTGGCCTCTTGCGAAGAGCAGCAAGA
GCTCGACAAGCGCTCAGAGGATGACGAGCCCAGTGCTATTGTTTTTGAA
//
NRC-114 Witch Flounder GC3.6 (SEQ ID NO: 112)
ATGAAGTTCACTGCCACCTTCCTGGTGTTGTTCATGGTCGTCCTCATGGCTGGATCCGGAGAGTGTGGTTGGAAAA
AGTGGCTCCGTAAAGGTAGAGTCATGGATTTAATTTGCTTTTTACATTGCAAATACTTTAATATAACATAGTTGGA
AAATCACAAAAATAAGTAGTCGATATATTTGGCCATATAGAATCACTTTGATTTCAATAATAATCAAAACAACAAT
CAAAAAGCCCATTGATTAGCATGTTCCTTCACTAAAATGGACATTGTCATTTATTTTGATTCTCACAGGCACCAAC
CTGCTGCGGCAACAATTGAAATCAAATTTGTCTCAGAAGAATTCAAAGTACATTGTTCTAGGCGATTTAATCTTTC
CATTCATCGGATTTGTTTTTAAAAATATAGAATAACTGGATCTCTATGTTAAAATAATAAAACACACATTCTGATT
TTACCTGTCAAGATTGAACACGACTTAAAAGTATGTATAAAACATCATCTGTATGTATAATTGTTTAACTGTCAAC
TAATAGTCCAAATAATTGTGTTATGGAAATGTATTCATTGTCATATAATATCATTTGCTTGAATTTATCACCATGT
GTTTTTGTTTGTTTTTACACAGGTGCCAAGCACCTTGGCCAGGCGGCCATTAAGTAAGGACTTCTACCATCATTAC
TGTGTAATTTTAACAGTATTATCATCAGTACTGTTATTGACAACTACTCTTGTCTCTGTGACTCTCTCCAGGGGTT
TGGCCTCTTGCGAAGAGCAGCAGGAGCTCGACAAGCGCTCAATGGATGACGAGCCCAGTGCTATTGTTTTTGACTG
AAGAAGTCGCCTTGAAGAGCCTTCAG
//
NRC-115 Witch Flounder GC2.2 (SEQ ID NO: 113)
GCCCACTTTGTATTCGCAAGGTAAGAGCGATATATTTCAAACTCATATAGACGAGACCAAGCATTTGGGAAATGTG
CTCAGCTTGTTACTGTATAATGCAAATGTTAACAATGTTTTTGTTCTGTTGTTTTTGCAGAATGAAGCTCGCTGCT
GCCTTCCTGGTGTTGTTCATGGTCGTCCTCATGGCTGAACATGGAGAGGGTTTTGGGGATTTCTATATGAAGCCTG
GTAGAGTCACGGAATTAATTCGATTTTAACATGGCAAATATTTTACTATAACATACCATATGAGTAGTCGATTAAT
TAATTGGATTTGTTTTTAAAAATATAGAATAATTGGATCTTTATGCTAAAATAATTAAACATACATTCTGATTTTA
CCAGTTAAGATTGAACGCTACTTAAAAGTATGTATAAAACATCATCTGTACATATAATTGTTTAACTGTTAACCAA
TAGTCCAAATAATTGTGTTGTGGAAATGTATTAATTGTCATTTAATATCATTTGCTTGAATTTGTCACCATGTGTT
GTTGTTTGTTTTTACACAGGTAGAAAGATTTCCCATGGGTAAGGACTTCTACCATCATTACTGTGTATTTTTAGCA
GTATTATCATCAGTACTGTTATTGATAACTTCTCTTGTCTCGCTGACTCTCTACAGGTACATCAGAAGTCCTTATG
GTTACGACGAGCAGCAGGAGGTCGACAAGCGCTCAGTCGATGACAACCCCAGTGCCATTGCTTCTGACTGAAGAAG
TCGCCTTGAAGGAGCCTTCAGA
//
NRC-116 Witch Flounder GcSc4B28 (SEQ ID NO: 114)
ATGAAGTTCACTGCCACCTTCCTGGTGTTGTTCATGGTCGTCCTCATGGCTGAACCTGGCGAGGGTTATTGGCGCT
TCCGCAACCACCGTGGTGAAAGGTTATCCCAGAGGCATTTCGCTGACGTCGAGCAGCAGGAGCTCGACAAGCGCTC
AGTGGATGACGAGCCCAGTTCTATTGCTTTTGA
//
NRC-117 Witch Flounder GC3.7 (SEQ ID NO: 115)
ATGAAGTTCACTGCCACCTTCCTCGTGTTGTTCATCGTCATGTTTGAACCTGGAGAGTGTTTTTGGAATGCTTTTT
CACCGGGTCCACCATGGTCGGGTCACGGAAGTAGTTCGATTTTTACATGGCAAATATTTAAATGAAACATACCATA
TGAGTAGTCGATATATTTGGCCAAGTAGAATCACTTTGACTTCAATAATAATCAAAAACATAATCAAAAAGCCCAT
TGATTAGCATGTTCCTTCAATGAAATGGACATTGAGGTTTATTTTGATTCTCACAGGCACCAACCTGCTGCGGCAA
CAATTGCATTCAAATTTGTCCCAAAGAAACTTAATTAACATTTTCTGGCGATTTAATCTTTGCATAAATTGGATTT
GTTTTTAAAAATATAGAATAACTGGATCTTTATGCTCAAATAATTAATCATACATTCTTATTTTATCAGTCAAGAT
TGAACGCTACTTAAAAGTATGTATAAAACATCATCTGTATGTATAATTGTTTAACTTTTAACTAAAAGTCCTAATA
ATTGTGTTATGGAAATGTATTAATTGTCATTTAATATCATTTCCTTGAATTTATCACCATGTGTTTTTGTTTGGTT
TTTACACAGCTGGAAGGTTGATCCATAGGTAAGGACTTCTACCATCATTACTGTATAATGTTAATAATAGCATTAT
CATCAGTACTGTTATTGATAACTTCTCTTGTCTCGCTGACTCTCTCCATCAGATTCATCAAACGTCACGGTGACGT
CGAGCAGCAGGAGCTCGACAAGCGCTCAGTGGATGACGAGCCCAGTTCTATTGCTTTTGCCTGAAGAAGTCGCCTT
G

//
NRC-118 Witch Flounder GC3.1 (SEQ ID NO: 116)
ATGAAGTTCACTGCCACCTTCCTGGTGTTGTTCATGGTCGTCCTCATGGCTGAACCTGGAGACTGTATTTTTGGAT
TGATTGCGACTGCGGTCCACAATGGTAAGTCAAGGAATTAATTCGATTTTTACGTGGCAAATATTTTAGTATAACA
TACCTTATGAGTAGTCGATATATTTGACCAAGTAGAATCATTTTGACTTCAATAATAATCAAAATAACAATCTCTA
GGCAATTTAATATTTGCATTAATTGGATTTGTTTTTAAAAATATAGAATAACTGGATCTTAATGCTAAAATAATTA
AACATACATTCTGATATTACCAGTCAAGATTGAACGCTACTTAAAAGTATGTATAAAACATCATCTGTATGTATAA
TTGTTTAACTGTCGACTAATAGTCCTAATAATTGTGTTATGGAAATGTATTCATTGTCATATAATATCATTTGCTT
GAATTTATCACCATGTGTTTTTGTTTGTTTTTACACAGCTGGAAGGTTGATCCATAGGTAAGGACTTCTACCATCA
TTACTGTATAATTTTAAGAGCATTATCATCAGTACTGTTATTGATAACTTCTCTTGTCTCGCTGACTCTCTCCATC
AGACTACTCGGCTTTCATCATGGGCCTCCCGGGTTCTGGCACGGTGACGTCGAGCAGCAGGAGCTCGACAAGCGCT
CAGTGGATGAGGAGCCCAGTTCTATTGCTTTTGACTGAAGAAGTCGCCTTGAAGGAGCCTTCAG
//
NRC-119 Witch Flounder GC4.1 (SEQ ID NO: 117)
ATGAAGTTCACTGCCACCTTCCTGGTGTTGTTCATGGTCGTCCTCATGGCTGAACCTGGAGACTGTATTTTTGGAT
TGATTGCGACTGCGGTCCACAATGGTAAGTCAAGGAATTAATTCGATTTTTACTTGGCAAATATTTTAGTATAACA
TACCTTATGAGTAGTCGATATATTTGACCAAGCAGAATCATTTTGATTTCAATAATAATCAAAATAACAATCTCTA
GGCAATTTAATATTTGCATTAATTGGATTTGTTTTTAAAAATATAGAATAACTGGATCTTAATGCTAAAATAATTA
AACATACATTCTGATATTACCAGTCAAGATTGAACGCTACTTAAAAGTATGTATAAAACATCATCTGTATGTATAA
TTGTTTAACTGTCGACTAATAGTCCTAATAATTGTGTTATGGAAATGTATTCATTGTCATATAATATCATTTGCTT
GAATTTATCACCATGTGTTTTTGTTTGTTTTTACACAGTTGGAAGGTTGGTCCATGGGTAAGGACTTCTACCATCA
TTACTGTATAATTTTAAGAGCATTATCATCAGTACTGTTATTGATAACTTCTCTTGTCTCGCTGACTCTCTCCATC
AGACTACTCGGCTTTCATCATGGGCCTCCCGGGTTCTGGCACGGTGACGTCGTGCAGCAGGAGCTCGACAAGCGCT
CAGTGGATGAGGAGCCCAGTGCTATTGTTTTTGAATGAAGAAGTCGCCTTGAAGGAGCCTTCAG
//
NRC-120 Witch Flounder GC4.4 (SEQ ID NO: 118)
ATGAAGTTCACTGCCACCTTCCTGGTGTTGTTCATGGTCGTCCTCATGGCTGAACCTGGAGACTGTATTTTTGGAT
TGATTGCGACTGCGGTCCACAATGGTAAGTCAAGGAATTAATTCGATTTTTACGTGGCAAATATTTTAGTATAACA
TACCTTATGAGTAGTCGATATATTTGACCAAGTAGAATCATTTTGGTTTCAATAATAATCAAAATAACAATCTCTA
GGCAATTTAATATTTGCATTAATTGGATTTGTTTTTAAAAATATAGAATAACTGGATCTTAATGCTAAAATAATTA
AACATACATTCTGATATTACCAGTCAAGATTGAACGCTACTTAAAAGTATGTATAAAACATCATCTGTATGTATAA
TTGTTTAACTGTCGACTAATAGTCCTAATAATTGTGTTATGGAAATGTATTCATTGTCATATAATATCATTTGCTT
GAATTTATCACCATGTGTTTTTGTTTGTTTTTACACAGTTGGAAGGTTGGTCCATGGGTAAGGACTTCTACCATCA
TTACTGTATAATTTTAAGAGCATTATCATCAGTACTGTTATTGATAACTTCTCTTGTCTCGCTGACTCTCTCCATC
AGACTACTCGGCTTTCATCATGGGCCTCCCAGGTTCTGGCACGGTGACGTCGAGCAGCAGGAGCTCGACAAGCGCT
CAGTGGATGAGGAGCCCAGTGCTATTGTTTTTGAATGAAGAAGTCGCCTTGAAGGAGCCTTCAG
//
NRC-121 Petrale sole 02A(3) (SEQ ID NO: 119)
ATGAAGTTCACTGCCACCTTCCTCGTGTTGTTCATGGTCATCGTCATGTTTGAACCTGGAGAGTGTTTTTTTGGAA
TGCGTTTTCACGGGGTCCACCATGGTAGGGTCACAAAAGTGATTTGATTATTACATGCCAAATATGTTAATGAAAC
ATACCATATGAGCAGTCGTATTATTTGGACAAGTAGAATCACTTTGATTTCAATAGTAATTAAAATAACAATCAAA
AAGGCCTTTGATTAGCATGTTCCTTCAATGAAATGGACATTGAGGTTTATTTTGATTCTCACCTGCATCGACCTGC
TGCGGCAACTATTGAAATCAAATTTGTCCCAGAAGAAACTAAATTAACATTTTCTAGGCCATCTAATCTTTGCATG
AATTGGATTTGCTTTCAAAAATATAGAATAACTGGATATTTATGCTAAAATAATAAAAACACACATTCTGATTTTA
CCAGTCAAGATTGAACACTACTTAAAAGTACGTATAAAACATCATCTGTATGTATAATTGTTTGACTTTTAACAAA
TAGTCAAAATGATTGTTATGGAAATGCATTAATTGTCATTTAATATCATTTACTTGAATTTATCACCATGTGTTTG
TTTGTTTTTTAGCAGGTGGAGGTTTTCTCAATGCGCAAGGACTTCTACCATCATTACTGTGTAATTTTAATAGTAT
TATCATCAGTACTCTTATTGACAACGTCTCTTGTCTCGCTGACTCTCTCTATCAGATTAAACCCAGGGTATCGCGG
TTACGACGAGCAGCAGGAGCTCGACAAGCGCGCAGTCGATGA
//
NRC-122 Petrale sole 02B (SEQ ID NO: 120)
ATGAAGTTCACTGCCACCTTCCTGGTGTTGTCCTTGGTCGTCCTCATGGCTGAACCTGGAGAGGGTTTCTTTGGAG
CCCTTCTCAAAGGTAGAGTCACGGAATTAATTTGATTGTTACATGGCAAATAATTTTGTATAACATATCATATGAG
CAGTCGATGTATTTGACCAAGAAGAATCATTTTGATTTCAATAATAATCAAAATAACAATCTCTTGGAGATTATAT
ATTTGCAATAATTGGATTTTATAAAATATAGAACAACTGGATCTTAATGCTAAAATAATTAAACATACATTCTGAT
TTTACCAGTCAAAATTAACCACTACTTTAAAGTATGTATAAAACATCATCTGTATGTTTAATTGTTTAACTTTTAA
CAAATAGTCCAAATAATTGTGTAATGGAAATGTATTCATTGTCATATAATATAGTTTGCTTGACTTTATCACCGTG
TGTTTTTGTTTGTTTTTTCACAGGTGCCCAGGCGCTCCATGGGTAAGGACTTCTACCATCATGACTGTGTAAGTTT
AATAATATTATCATCAGTACTGTTATTAACGACTTCTCTTGTCTCGCTGACTCTCTCCATCAGAATCATCCACAAT
GCTCGTCACGGTTACGACGAGCAGCAGGAACTCAACAAGCGCGCAGTCGATGA
//
NRC-123 Petrale sole PL1/2/2.1 (SEQ ID NO: 121)
GCCCACTTTGTATTCGCAAGGTAAGATCAATATTTTTCAAATTCATTTAGACGAGACCAACCGTTTGCGAAATGTG
CTCAGCTTGTTATTGTATAATAACAAAGTTAACGATCTTTATTTTTCTGTTTTTTTGTAGAATGAAGTTCACTGCC

ACCTTCCTGATGTTGTTCATCTTCGTCCTCATGGTTGAACCTGGAGAGTGTGGTTGGAAAGATTGGTTTCGTAAGG
CTAAGAAAGGTAGAATCACGGAATTAATTAGCTTTTTACATTGCAAATAGATTTTTTATAACAGCTGGAAATCACA
AAAATAAATAGTCGATATATTTGGCCAATTAGAATCACTTTAATTTCAATAATAATCTAAATAACAACCTAAAAGG
CCTTTGATTAGCATGTTCCTTCAATGAAAAGGACATTGAGGTTTATTTTGATTCTCACATGCACCGACCTGTGCGG
CAACAATTGAATTCAGATTTGTCCCAGAAGAATTCAAAGTACATTTTTCCAGGCGATTAAATCTTTCCATTACTCG
GATTTAAAAATAAATAAATAGAATAACTGAAGCGCTATGATAAAATAATTACACATTCATTCTGATTTTACAAGTC
AAGATTGAACACTATTAAAAAGTGTGTATAAAACATCATCTGTATGTATAATTGTTTAACTGTTAATAGTCTTAAT
AATTGTGTTATGGAAATGTATTAATTTACATTTAATATCATTTGCTTGAGTTTACCATCATGTGTTTTTGTTTGTT
TTTACACAGTTGGCAAGACTGTTGGCGGCTTGGCCCTTAAGTAAGAACTTCTACCATCATTACTGTATAATTTTGA
TAGTATTATCACCAGTACTGTTATTAACTACTTCTCTTGTCTCGCTGACTCTCTCCATCCGACTCATCCGCAGTCA
TTACCTTGGCGAGCAGCAGGAGCTTGCCAAGCGCGCAGTCGATGACGACCCCAGTGTTATTGTCTTTGACTGAAGA
AGTCGCCTTGAAGGAGCCTTCAG
//
NRC-124 English sole 05A (SEQ ID NO: 122)
ATGAAGTTCACTGCCACCTTCCTCATGATTTTAATCTTCGTCCTCATGGTCGAACCTGGAGAGTGTGGTATTAGGA
AATGGTTTAAAAAGGCTGCTCACGGTAAAGTCACGGAATTAATTTGCTTTTTGCTTTACAAATATTTTTTTATAGC
AGCTGGAAAATCACAAAAATAAATAGTCGATGTATTTGGCCAATTAGAATCACTTTGATTTCAAATAATAATCTAA
ATAGCAACCTAAAAGGCCTTTGATTAGCATGTTCCTTCAATGAAATGGATGTTGAGGTTTATTTTGATTCTCACAT
GCACCGACCTGCTGCGGCAACAATTGAATTCAAATTTGTCCCAAAGGAATTCAAAGTAAACTTTTCTAGATGATTT
AATCTTTCCATAACTCGGCTTTGTTTTTAAAAATATATAATAACTCAATCACTATGATAAAATAATAACACATACA
TTCTGATTTATACAAGACAAGATTGAAAACTTCTTAAAAGTATGTATAAAACATCATCTGTTTGTATAATTGTTTA
TCATTTCACAAAAAGTCCAACTAATTGTGTTATGGAATTGTATAAATTGTCATTTAATATAATTTTTTTGAGTTTA
TCAATATGTGTTTTTGTTTGTTTTACACAGTTGGCAAGGAAGTTGGCAAGGTGGCCCTTAAGTAAGGACTTCTACC
ATTATTACTGTATAATTTTGATAGTATTATCACCCGTACTGTTATTGACAACTTCTCTTTTCCTGCTGACTCTCTC
CATCTGACTCATCTGCAGTGCTTGCCTTGACAAGCAGCAGCAGCTCGACAAGCGCGCAGTCGATGA
//
NRC-125 English sole PL1/2/5 (SEQ ID NO: 123)
GCCCACTTTGTATTCGCAAGGTAATATCGATATTTTTCAAACTCATTTAGACGAGACCAAGCATTTGGGAAATGTG
CTAAGGTTGTTACTGTATAATGCAAAATTAATGATCTTTATTTTTCTGTTTTTTTTTTGCAGAATGAAGTTCACTGC
CACCTTCCTCATGATTTTAATCTTCGTCCTCATGGTCGAACCTGGAGAGTGTGGTTTGAAGAAATGGTTTAAAAAG
GCTGTTCACGGTAGAGTCACGGAATTAATTTGCTTTTTGCTTTACAAATATTTTTTTTATAGCAGCTGGAAAATCAC
AAAAATAAATAGTCGATGTATTTGGCCAATTAGAATCACTTTGATTTCAATAATAATCTAAATAGCAACCTAAAAG
GCCTTTGATTAGCATGTTCCTTCAATGAAATGGATGTTGAGGTTTATTTTGATTCTCACATGCACCGACCTGCTGC
GGCAACAATTGAATTCCAATTTGTCCCAAAGGAATTCAAAGTAAACTTTTCTAGGCGATTTAATCTTTCCATAACT
CGGCTTTGTTTTTAAAAATATATAATAACTCAATCCCTATGATAAAATAATAACACATACATTCTGATTTATACAA
GACAAGATTGAAAACTTCTTGAAAGTATGTATCAAACATCATCTGTTTGTATAATTGTTTAACAGTTCACAAAAAG
TCCAACTAATTGTGTTATGGAATTGTATAAATTGTCATTTAATATAATTTTTTTGAGTTTATCAATATGTGTTTTT
GTTTGTTTTACACAGTTGGCAAGAAAGTTGGCAAGGTGGCCCTTAAGTAAGGACTTCTACCATTATTACTGTGTAA
TTTTGATAGTATTATCACCAGTACTGTTATTGACAACTTCTCTTTTCCTGCTGACTCTCTCCATCCGACTCATCTG
CAGTGCTTACCTTGGCGAGCAGCAGCAGCTCGACAAGCGTGCAGTCGATGAAGAGCCCAGTGTTATTGCTTTTGAC
TGAAGAAGTCGCCTTGAAGGAGCCTTCAG
//
NRC-126 Starry flounder 09A (SEQ ID NO: 124)
ATGAAGTTCACTGCCACCTTCCTCATGATGTTCATCTTCGTCCTCATGGTTGAACCTGGAGAGTGTGGTTGGAGGA
AATGGATTAAAAAGGCTACTCACGGTAAAGTCACGGAATTAATTCGTTTTTTGCTTTGCAAATATTTTTTTTATAA
CAGCTGGAAAGTCACAAAAATAAATAGTCAATATATTTGGCCAATTAGAATCACTTTGAGTTCAATAATAATCTAA
ATAACAACCAAAAAGGCCTTTCCTTTAATGAAATGTACGTTGAAGTTTATTTTGAATCTCACATGCACCGACCTGC
TGCGGCAACAATTGAATTCAAATTTCTCCCAGAGGAATTCAAAGTAAATTTTTCTAGGCGATTTAATCTTTCCATT
ACTCTGATTTGTTTTAAATATATAGAATGACTCAATTGCTATGATAAAATAATAAGCCATACATTCTGATTTTTAC
AAGACAAGATTGAAAACTTCTTAAAAGTACGTATAAAACATCATCTGTATTTATAATTGTTTAACATTTAACAAAT
TGTCCTACTAATTGTGTTATGGAAATGTATAAATTGTCATTTAATATCATTTGCTTGAGTTTATCATTATTTGTTT
TTGTTTGTTTTTACACAGTTGGCAAGCATATTGGCAAGGCGGCCCTTGAGTAAGAACTTCTACCATCATTACTGTA
TAATTTTGATAGTATTATCACCAGTACTGTTATTGACAACTTCTCTTGTCCTGATGACTCTGTTCATCCAACTCAT
CTGCAGTGCTTACATTGGCGGGAAGCAAGAACTCGACAAGCGCGCAGTCGATGA
//
NRC-127 (SEQ ID NO: 327)
ATGAAGTTCACTGCCACCTTCCTCATGATTTTAATCTTCGTCCTCATGGTCGAACCTGGAGAGTGTGGTTGTAAGA
AATGGTTTAAAAAGGCTGCTCACGGTAGAGTCACGGAATTAATTTGCTTTTTGCTTTACAAATATTTTTTTTATAGC
AGCTGGAAAATCACAAAAATAAATAGTCGATGTATTTGGCCAATTAGAATCACTTTCATTTCAATAATAATCTAAA
TAGCAACCTAAAAGGCCTTTGATTAGCATGTTCCTTCAATGAAATGGATGTTGAGGTTTATTTTGATTCTCACATG
CACCGACCTGCTGCGGCAACAATTGAATTCCAATTTGTCCCAAAGGAATTCAAAGTAAACTTTTCTAGGCGATTTA
ATCTTTCCATAACTCGGCTTTGTTTTTAAAAATATATAATAACTCAATCCCTATGATAAAATAATAACACATACAT
TCTGATTTATACAAGACAAGATTGAAAACTTCTTGAAAGTATGTATCAAACATCATCTGTTTGTATAATTGTTTAA
CATTTCACAAAAAGTCCAACTAATTGTGTTATGGAATTGTATAAATTGTCATTTAATATAATTTTTTTGAGTTTAT

CAATATGTGTTTTTGTTTGTTTTACACAGTTGGCAAGAACGTTGGCAAGGTGGCCCTTAAGTAAGGACTTCTACCA
TTATTACTGTATAATTTTGATAGTATTATCACCAGTACTGTTATTGACAACTTCTCTTTTCCTGCTGACTCTCTCC
ATCCGACTCATCTGCAGTGCTTACCTTGGTGAGCAGCAGCAGCTCGACAAGCGTGCAGTCGATGAAGAGCCCAGTG
TTATTGCTTTTGACTGAAGAAGTCGCCTTGAAGGAGCCTTCAG

//

NRC-128 (SEQ ID NO: 129)

GCCCACTTTGTATTCGCAAGGTAATATCGATATTTTTCAAACTCATTTAGACGAGACCAAGCATTTGGGAAACGTG
CTAAGGTTGTTACTGTATAATGCAAAATTAATGATCTTTATTTTTCTGTTTTTTTTTTGCAGAATGAAGTTCACTGC
CACCTTCCTCATGATTTTAATCTTCGTCCTCATGGTCGAACCTGGAGAGTGTGGTATTAGGAAATGGTTTAAAAAG
GCTGCTCACGGTAAAGTCACGGAATTAATTTGCTTTTTGCTTTACAAAATATTTTTTTATAGCAGCTGGAAAATCA
CAAAAATAAATAGTCGATGTATTTGGCCAATTAGAATCACTTTGATTTCAATAATAATCTAAATAGCAACCTAAAA
GGCCTTTGATTAGCATGTTCCTTCAATGAAATGGATGTTGAGGTTTATTTTGATTCTCACATGCACCGACCTGCTG
CGGCAACAATTGAATTCAAATTTGTCCCAAAGGAATTCAAAGTAAACTTTTCTAGGCGATTTAATCTTTCCATAAC
TCGGGCTTTGTTTTTAAAAATATATAATAACTCAATCCCTATGATAAAATAATAACACATACATTCTGATTTATAC
AAGACAAGATTGAAAACTTCTTGAAAGTATGTATCAAACATCATCTGTTTGTATAATTGTTTAACATTTCACAAAA
AGTCCAACTAGTTGTGTTATGGAATTGTATAAATTGTCATTTAATATAATTTTTTTGAGTTTATCAATATGTGTTT
TTGTTTGTTTTACACAGTTGGCAAGAAAGTTGGCAAGGTGGCCCTTAAGTAAGGACTTCTACCATTATTACTGTAT
AATTTTGATAGTATTATCACCAGTACTGTTATTGACAACTTCTCTTTTCCTGCTGACTCTCTCCATCCGACTCATC
TGCAGTGCTTACCTTGGCGAGCAGCAGCAGCTCGACAAGCGTGCAGTCGATGAAGAGCCCAGTGTTATTGCTTTTG
ACTGAAGAAGTCGCCTTGAAGGAGCCTTCAG

//

NRC-129 (SEQ ID NO: 130)

AATGAAGTTCACTGCCACCTTCCTCATAGAATGGTTCATCTTCGTCCTCAATGGGTTGAAACCTGAAGAAGTGTGG
TTGGAAAGAAAGTGGTTTAAAAAGGCTACTCACGGTAAAGTCACGGAATTAATTAGCATTTTTCTTTGCAAATATT
TTTTTTATACAGCTCGAAAATTCACAAAAATAAATAGTCGATATATTTGGCCAATTAGAATCACTTTGATTTCAAT
AATAATCTAAATAACAACCTAAAAGGCCTTTGATTAGCATGTTCCTTCAATGAAATGGACGTTGAGGTTTATATTG
ATTCTCACATGCACCGACCTGCTGCGTCAACAATTGAATTCAAATTTGAGAGGAATTCAGCGTAAATTTTTCTAGG
CGATTTAATCTTTCCATTACTCGGATTTGTTTTTAAATATATAGAATAACTCAATTGCTATGATAAAATAATAACA
CATACATTCAGATTTTTACAAGACAAGATTGAAAACTTCTTAAAGGTACGTATAAAACATCATCTGTATTTATAAT
TGTTTAACATTTAACAAATAATCCTACTAATTGTGTTATGGAAATGTATAAATTGTAATTTAATATAATTTGCTTT
AGTTTATCATTATTTGTTTTTGTTTGTTTTTACACAGTTGGCAAGCATGTTGGCAAGGCGGCCCTTGAGTAAGAAC
TTCTACCATCATTACTGTATAATTTTGATAGTGTTATCACCAGTACTGTTATTGACAACTTCTCTTGTCCTGCTGA
CTCTCTCCATCCGACTCATCCGCAGTGCTTACCTCGGCGAGAAGCAAGAACTCGACAAGCGCGCAGTCGATG

//

NRC-130 Greenland halibut 12B (SEQ ID NO: 131)

ATGAAGTTCACTGCCACCTTCCTGGTGTTGTTCATGGTCGTCCTCATGGCTGAACCTGGAGAGGGTTTTTTCGGAT
TGCTTTTTCACGGGATCCACCATGGTAGGGTCACGGAATTAATTAGATGTTTACATGGCAAATATTTTAAGATAAC
ACACCATATGAGTAGTCGATATATTTGACCAATTAGAATCACTTTAATTTCAATAATAATCACAATAACAATCTCT
AGGCCATTTAATCTTTCCATTAATCGGATTTGTTTTTTTAAATATAGAATAACTGGATCTTTATGCTAAAATAATG
AAACATACATTCTGATTTTACCAGTCAAGATTGAACGTTACTTAAAAGTATGTTTAAAACATCATCTGTATGTATA
ATTGTTTAGCTGTAAACAAATAGTCCAAATAATTGTGTTATGGAAATGTATTAATTGTCATATAATATAATTTGCT
TGAATTTATCACCATGTGTTTTTGTTTGTTTTTTAACACAGCTGGAAAGTTGATCCATGGGTAAGGACTTCTACCA
TCATTACTGTGTATTTTTAATAGTATTATCATCAGTACTGTTATTAACAACTTCTCTTCTATCGCTGACTCTCTCC
ATCAGACTCATCCATCATGGTTACGACGAGCAGCAGGAGCTCGACAAGCGCGCAGTCGATGA

//

NRC-131 Pacific Halibut 15A (SEQ ID NO: 132)

ATGAAGTTCACTGCCACCTTCCTGGTGTTGTTCATGGTCGTCCTCATGGCTGAACCTGGAGAGGGTTTGGGAAATT
GGATGGGGCCCCATATCAGCGGTAGAGTCACGGAATTAATTTGCTTTTTCCATTGCAAATATTTTAATATTGCATA
GCTGGAAAATCACGAAATAAGTAGTCGATATATTTGGCCAAATAGAATAACTTTGATTTCAATAATAATCAAAATT
ACAATCAAAAAGGCCTTTGATTAGCATGTTCCTTCAATAAAATGGACATTGAAGTTTATTTTGATGCTCACATGCA
CCGACCTGCTGCGGCAACAATTGAAATCAAATTTGTCTCAGAATTTAAAGTACATTTTTCTAGGTGATTTAATCTT
TCCATTCATCTGATTTATTTTATAAATATAGAATAACTGGATCTTTCTGCTAAAATAATAAAACACACATTCTGAT
TTTACCAGTCAAGATTGAACACTACTTAAAAGTATGTATAAAACATCATCTGTATGTATAATTGTTTAACTGTTAA
CAATAGTCCAAATAATTGTGTTAAGGAAATGTATTAATTGTCATTTAATATCATTTGCTTGAATTTATCACCATGA
GTTTTTTGTTTGTTTTTACACAGGTAGAAAGAAGGCCTTGCAGTAAGGACTTCTACCATCATTACTTTGTAATTTT
TATAGTATTATCATCAGTACTGTTATTGACAACTTCTCTTGTCTCGCTGACTCTCTCCATCAGGATGAACTCAGAG
CGTCGCAGTTACGACGAGTAGCAGCAGAAGCTCGACAAGCGCGCAGTCGATGA

//

NRC-132 Pacific Halibut 15B (SEQ ID NO: 133)

ATGAAGTTCACTGCCACCTTCCTGGTGTTGTTCATGGTCGTCCTCATGGCTGAACCTGGAGAGTGTTTTTTGGGAT
TGCTTTTTCACGGGGTCCACCATGGTAGGGTCACGGAAGTAATTCGATTTTTACATGGCAAATATTTTAAGATAAC
ACACCATATGAGTAGTCGATATATTTGATATATTAGAATCACTTTGATTTCAATAATAATCAAAATAACAATCTCT
AGGCGATTTAATATTTGCATTAATTGGATTTGTTTTTAAAAATATAGAATAACTGGATCTTTATGGTAAAATAATT

AAACATACATTCTGATTTTACCAGTCAAGATTGAACACTACTTAGAAGTATGTATAAAACATCATCTGTATGTATA
ATTGTTTAACTGTTAACTAATAGTCCAAATAATTGTGTTATGGAAATGTATTAATTGTCATTTAATATCATTTGCT
TGAATTTATCACCATGTGTTTTTGTTTGTTTTTACACAGTTGGAAATTTGATCCATGGGTAAGGACTTCTACCATC
ATTACTGTGTATTTTTAATAGTATTATCATCAGTACTGTTATTGACAACTTCTCTTGTCTCGCTGACTCTCTCCAT
CAGACTCATCCATCACGGTTACGACGAGCAGCAGGAGCTCGACAAGCGCGCAGTCGATGA
//
NRC-133 C O sole PL1/2/6 (SEQ ID NO: 134)
GCCCACTTTGTATTCGCAAGGTAATATCGATATTTTTCAAACTCATTTAGACGAGACCAGGCATTTGGGAAACGTGC
TAAGGTTGTTACTGTATAATGCAAAATTAATGATCTTTATTTTTCTGTTTTTTTTTTGCAGAATGAAGTTCACTGCCA
CCTTCCTCATGATTTTAATCTTCGTCCTCATGGTCGAACCTGGAGAGTGTGGTATTAGGAAATGGTTTAAAAAGGCT
GCTCACGGTAAAGTCACGGAATTAATTTGCTTTTTGCTTTACAAATATTTTTTTACAGCAGCTGGAAAATCACAAAA
ATAAATAGTCGATGTATTTGGCCAATTAGAATCACTTTGATTTCAATAATAATCTAAATAGCAACCTAAAAGGCCTT
TGATTAGCATGTTCCTTCAATGAAATGGGTGTTGAGGTTTATTTTGATTCTCACATGCACCGACCTGCTGCGGCAAC
AATTGAATTCAAATTTGTCCCAAAGGAATTCAAAGTAAACTTTTCTAGGCGATTTAATCTTTCCATAACTCGGCTTT
GTTTTTAAAAATATATAATAACTCAATCGCTATGATAAAATAATAACACATACATTCTGATTTATACAAGACAAGAT
TGAAAACTTCTTGAAAGTATGTATCAAACATCATCTGTTTATATAATTGTTTAACATTTCACAAAAAGTCCAACTAA
TTGTGTTATGGAATTGTATAAATTGTCATTTAATATAATTTTTTTGAGTTTATCAATATGTGTTTTTGTTTGTTTTA
CACAGTTGGCAAGAAAGTTGGCAAGGTGGCCCTTAAGTAAGGACTTCTACCATTATTACTGTATAATTTTGATAGTA
TTATCACCAGTACTGTTATTGACAACTTCTCTTTTCCTGCTGACTCTCTCCATCCGACTCATCTGCAGTGCTTACCT
TGGCGAGCAGCAGCAGCTCGACAAGCGTGCAGTCGATGAAGAGCCCAGTGTTATTGCTTTTGACTGAAGGAGTCGCC
TTGAAGGAGCCTTC
//

Table 13 Nucleotide sequences encoding hepcidin-like peptides of Table 11.

NRC201 (SEQ ID NO: 135)
CGCCCTTAAGATGAAGACATTCAGTGTTGCAGTTGCAGTGGTGGTCGTCCTCGCATGTATGTTCATCCTTG
AAAGCACCGCTGTTCCTTTCTCCGAGGTGCGAACGGAGGAGGTTGAAAGCATTGACAGTCCAGTTGGGGAA
CATCAACAGCCGGGCGGCACGTCCATGAATCTGCCGGTACGTTCAATTTAGTGAATGAATTAAGTAATTAC
CTTTAGCAAATTAACATCTAAGTGGTTGCGTTTCACCCTTGGAATTGAATTAGCCCACTAGCGCTAGTTGT
TAACCATTTGATTGTGAGCCGGTAGAGAGGGCTTCAGGGCGAGTAGTGTGAATACTTGTGAAGTGGAGACT
TGGACAAAAATACTTACCATGTGCTTGTTCCCACCTTTTTCATTTTCTTTTCTTGGCTGAGATACAGATGC
ATTTCAGGTTCAAGCGTCAGAGCCACCTCTCCCTGTGCCGTTGGTGCTGCAACTGCTGTCACAACAAGGGC
TGTGGCTTCTGCTGCAAATTCTGAGGACCTGCCAGCAAAGGGCGAATTCGTTTAAAACAC
//
NRC202 (SEQ ID NO: 136)
AGATGAAGACATTCAGTGTTGCAGTTGCAGTGGTGGTCGTCCTCGCATGTATGTTCATCCTTGAAAGCACC
GCTGTTCCTTTCTCCGAGGTGCGAACGGAGGAGGTTGAAAGCATTGACAGTCCAGTTGGGGAACATCAACA
GCCGGGCGGCACGTCCATGAATCTGCCGATGCATTTCAGGTTCAAGCGTCAGAGCCACCTCTCCCTGTGCC
GTTGGTGCTGCAACTGCTGTCACAACAAGGGCTGTGGCTTCTGCTGCAAATTCTGAGGACCTGCCAGCA
//
NRC203 (SEQ ID NO: 137)
ACGAGGTCCCTCATCCGCTGACACCAAAAGAACAATCAATCAACTTTGGACTCGTCTTAGTGCATTGAAAA
TTGTGCGTTGGAGAGCGTCGCTTTTTGGGAACATTGAAGAGTTCTGATCTTCCTCATAAACTGTCACTTCA
ATTTCAACTGATTTCAACAGGACTTTTAAATAGGCTATAAACTTCCTAAAAAAAAACGAGAATGAAGGCCTT
TAGTGTTGCAGTGGTACTCGTCATTGCATGTATGTTCATCCTTGAAAGCACCGCTGTTCCTTTCTCCGAGG
TGCGAACGGAGGAGGTTGGAAGCTTTGACAGTCCAGTTGGGGAACATCAACAGCCGGGCGGCGAGTCCATG
CATCTGCCGGAGCCTTTCAGGTTCAAGCGTCAGATCCACCTCTCCCTGTGCGGTTTGTGCTGCAACTGCTG
TCACAACATTGGCTGTGGCTTCTGCTGCAAATTCTAAGGACCTGCCCGCAACATTTTCTAGTTTGTACATG
TTTGCAATGTTTTCTTTCTGAGATGTTGTTTTTGTGACTATGATAATGATTTATAAAATCACT
TCTTATTGTGACACTTTAAAAAAAATAAACACATTCTTTGAATACAAAAAAAAAAAAAAAAAA
//
NRC204 (SEQ ID NO: 138)
CGAACGGAGGAGGTTGAAAGCATTGACAGTCCAGTTGGGGAACATCAACAGCCGGGCGGCACGTCCATGAA
TCTGCCGATGCATTTCAGGTTCAAACGTCAGAGCCACCTCTCCCTGTGCCGTTGGTGCTGCAACTGCTGTC
ACAACAAGGGCTGTGGCTTCTGCTGCAAATTCTGAGGACCTGCCAGCACTAAAGCCATTTTATTAACTTAT
CGCCTTTAATTTGCCCCTATTCTTCTATGTTTCTTTTGGACTCTGTGGAGAAGATGCAATCTCATTGACGT
CTTTATCACTGCACAACCTCAATCTTGT
//
NRC205 (SEQ ID NO: 139)
AAGATGAAGACATTCAGTGTTGCAGTGGTACCCGTCATTGCATGTATGTTCATCCTTGAAAGCACCGCTGT
TCCTTTCTCCGAGGTGCGAACGGAGGAGGTTGGAAGCTTTGACAGTCCAGTTGGGGAACATCAACAGCCGG
GCGGCACGTCCATGAATCTGCCGATGCATTTCAGGTTCAAGCGTCAGAGCCACCTCTCCCTGTGCCGTTGG
TGCTTCAACTGCTGTCACAACAAAGGCTGTGGCTTCTGCTGCAAATTCTGAGGACCTGCCAGCA
//
NRC206 (SEQ ID NO: 140)
TAAGATGAAGCAATTCAGTGTGGCAGTGGTACTCGTCATGGCATGTATGTTCATCGTGGAAAGCACCGCTG
TTCCTTTCTCCGAGGTGCGAACGGAGGAGGTTGGAAGCTTGGACAGTCCAGTTGGGGAACATCAACAGCCG
GGCGGCGAGTCCATGCATCTGCCGGAGCCTTTCAGGTTCAAGCGTCAGATCCACCTCTCCCTGTGCGGTTT
GTGCTGCAACTGCTGTCACAACATTGGCTGTGGCTTCTGCTGCAAATTCTGAGACTGCCAGCA
//
NRC207 (SEQ ID NO: 141)
ACGAGGCACACGCTGACCAGGGGGTCACCACAACTTCTGAAGAGACCCAGGTTCCTAGAGAGCCACTAGAG
AATCACCCGGGAGCCCGAAGAACACAGGACGCTGCGGTGCTCGTCGGTGGCCGGACACCCATGAGACAGAA
GACCTACAAGCCTCTCAGCTTCAGAAGGATTTCCTGACTCAGCATCTAAAACCTCCCTCAAAATGAAGGCA
TTCAGCATTGCAGTTGCAGTGACACTCGTGCTCGCCTTTGTTTGCATTCAGTGCAGCTCTGCCGTCCCATT
CCAAGGGGTGCAGGAGCTGGAGGAGGCCGGGGGCAATGACACTCCAGTTGCGGAACATCAAGTGATGTCAA
TGGAATCCTGGATGGAGAATCCCACCAGGCAGAAGCGCCACATCAGCCACATCTCCCTGTGCCGCTGGTGC
TGCAACTGCTGCAAGGCCAACAAGGGCTGTGGCTTCTGCTGCAAGTTCTGAGGATTCCCGCAACACAACCT
CACAATGTATTAATTTATTACACTTTTTGTCGAGAAATGTCCTTTTTCTTGACCTCTTTTGTAATTTTGTA
TAATCTTTTAAATAAAACGGGGTACGATTCATGGAAAAAACCCTTTGAATAAAATAAAAAAAAAAAAAAAA
AAAAAAAC
//
NRC208 (SEQ ID NO: 142)
AAGATGAAGACATTCAGTGTTGCAGTTGCAGTGACACTCGTGCTCGCCTTTGTTTGCATTCAGGACAGCTC
TGCCGTCCCATTCCAGGGGGTAAGAACGCAACTTTAACTCGCTTCATTTGCTTATTAGCCATAAATGTTTT
GTCAGGATGCTGAGACACGGCTCCTAAATGTGTATAATTCATTAACAGGTGCAGGAGCTGGAGGAGGCAGG

GGGCAATGACACTCCAGTTGCGGCACATCAAATGATGTCAATGGAATCGTGGATGGTATGTTCAATCTGTT
CAATCGACTGGATGAATTAAGCCAATTACTGTGAGCGCGTTAACATTTAAGTGGCTGTGTTCCAGCCCGGT
GCTGTAGGGAATAAAACCCCTCGTTCATGTGTCTTGTCCGTCCACAGGAGAGTCCCGTCAGGCAGAAGCGT
CACATCAGCCACATCTCCATGTGCCGCTGGTGCTGCAACTGCTGCAAGGCCAAGGGCTGTGGCCCCTGCTG
CAAATTCTGAGGACCTGCCCAGCA
//
NRC209 (SEQ ID NO: 143)
AAGATGAAGACATTCAGTGTTGCAGTCACAGTGGCCGTCGTGCTCGTCTTTATTTGTATCCAGCAGAGCTC
TGGCACCTTTCCTGAGGTAAGCTCCTGACTTCAGATCGTTTCATTTTGCTTGTTATCCATGAATCTCTCAT
CAACAGACTGAGACTTGATTCCTTCTTTATCAGGTACAAGAGCTGGAGGAGGCAGTGAGCAATGACAATGC
AGCTGCTGAACATCAGGAGACATCAGTGGACTCATGGATGGTAGGTTCAGTTCACTGAATGGATCAAACCA
ATTCACATCAGACCTTTCAGATGGAAGTGAATGTGTTTTAGTCTCAAAGGTGCCCTGAAGCTCAGTTTACA
CAAGCAGTGAAAACAAACACAGAAAGTTATGATGATGCTGATGAACTTCTCCTCATGTCTCATGTCTCTCA
CACAGATGCCATACAACAGACAGAAGCGTGCCTTCAAGTGTAAGTTCTGCTGCGGCTGCTGCAGAGCTGGT
GTCTGTGGACTGTGCTGCAAGTTCTGAGGATTCCTGCTCCAACAAC
//
NRC210 (SEQ ID NO: 144)
ACGAGCTGACAGGAGCTGACAGGAGTCACCAGCAGAGTCAAAGAACTAAACAACTTAACTCAGTCAAACTC
TCAAAGATGAAGACATTCAGTGTTGCAGTCACAGTGGCCGTCGTCCTCGTCTTTATTTGTATCCAGCAGAG
CTCTGCCTCCTTTCCTGAGGCACAAGAGCTGGAGGAGGCAGTGAGCAATGACAATGCAGCTGCTGAGCATC
AGGAGACACCAGTGGACTCGTGGATGATGCCATACAACAGACAGAAGCGTAGCTTTAAGTGTAAGTTCTGC
TGCGGCTGCTGCAGAGCTGGTGTCTGTGGACTGTGCTGCAAGTTCTGAGGATTCCTGCTCCAACAACCATC
AAATATTCATTTGTTTTGCCTTTTGTCTTAAAGTTCATTGAACTATAAACATATTTCTGGTTGAGCATGTG
ATAGTTTAATGGTGTTACTCATTGGTTCATGGTATAGTCAAGTGTTCAGAGATGTGATTGTATCACCCACA
TATTTTCTCTGTTAGGTGTATTTTCAATAAATGCCAATGATCCTTTGAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAA
//
NRC211 (SEQ ID NO: 145)
ACGAGCGGCACGAGGTGAACTGACAGGAGCTGACAGGAGTCACCAGCAGAGTCAAAGAACTAAACAACTTA
ACTCAGTCAAACTCTCAAAGATGAAGACATTCAGTGTTGCAGTCACAGTGGCCGTCGTGCTCGTCTTTATT
TGTATCCAGCAGAGCTCTGCCTCCTTTCCTGAGGCACAAGAGCTGGAGGAGGCAGTGAGCAATGACAATGC
AGCTGCTGAACATCAGGAGACACCAGTTGACTCGTGGATGATGCCAAACAACAGACAGAAGCGTGGCTTTA
AGTGTAAGTTCTGCTGCGGCTGCTGCAGAGCTGGTGTCTGTGGACTGTGCTGCAAGTTCTGAGGATTCCTG
CTCCAACAACCATCAAATATTCATTTGTTTTGCCTTTTGTTTTAAAGTTCATTGAACTATATACATATTTC
TGGTAGAGCATGTGATAGTTTAATGGTGCTACTCCTTGGTTCATGGTGTAGTTAAGTGTTCAGAGATGTGA
TTGTATCACCCACATATTTCTCTGTTAAGGTGTATTTTCAATAAATGTTAATGCTCCTTTGAAAAAAAAAA
AAAAAAAAAAA
//
NRC212 (SEQ ID NO: 146)
ACGAGACTGACAGGAGCTGACAGGAGTCACCAGCAGAGTCAAAGAACTAAACAACTTAACTCAGTCAAACT
CTCAAAGATGAAGACATTCAGTGTTGCAGTCACAGTGGCCGTCGTGCTCGTCTTTATTTGTATCCAGCAGA
GCTCTGCCACCTTTCCTGAGATGCCATACAACAGACAGAAGCGTGGCTTTAAGTGTAAGTTCTGCTGCGGC
TGCTGCGGAGCTGGTGTCTGTGGAATGTGCTGCAAGTTCTGAGGATTCCTGCTCCAACAACCATCAAATAT
TCATTTGTTTTGCCTTTTGTCTTAAAGTTCATTGAACTATAAACATATTTCTGGTTGAGCATGTGATAGTT
TAATGGTGTTACTCATTGGTTCATGGTATAGTCAAGTGTTCAGAGATGTGATTGTATCACCCACATATTTT
CTCTGTTAGGTGTATTTTCAATAAATGCCAATGATCCTTTGAAAAAAAAAA
//
NRC213 (SEQ ID NO: 147)
AAGATGAAGACATTCAGTGTTGCAGTCACAGTGGCCGTCGTGCTCGTCTTTATTTGTATCCAGCAGAGCTC
TGCCTCCTTTCCTGAGGTAAGCACCTGACTTCAGATCGTTTCATTTGCTTGTTATCCATGAATCTCTCATC
ATCATACTGAGACTTGATTCCTTCTTTATCAGGCACAAGAGCTGGAGGAGGCAGTGAGCAATGACAATGCA
GCTGCTGAGCATCAGGAGACACCAGTGGACTCCAGGAGTGAATGTGTTTTAGTCACAAAAGTGCCCTGAAG
CTCAGTTTACACAAGCAGAGAAAACAAACAGAGTAAGTTATGATGATGCTGATGAAGGTCTCCTCATGTCT
CATGTCTCTCACACAGATTCCATACAACAGACAGAAGCGTAGCTTTAAGTGTAAGTTCTGCTGCGGCTGCT
GCAGAGCTGGTGTCTGTGGACTGTGCTGCAAGTTCTGAGGATTCCTGCTCCAACAAC
//
NRC214 (SEQ ID NO: 148)
AGATGAAGACATGCAGTGTTGCAGTCACAGTGGCCGTCGTGCTCGTCTTTATTTGTATCCAGCAGAGCTCT
GCCTCCTTTCCTGAGGTAAGCACCTGACTTCAGATCGTTTCATTTGCTTGTTATCCATGAATCTCTCATCA
TCATACTGAGACTTGATTCCTTCTTTATCAGGTACAAGAGCTGGAGGAGGCAGTGAGCAATGACAATGCAG
CTGCTGAACATCAGGAGACACCAGTTGACTCGTGGATGGTAGGTTCAGTTCACTGAATGGATCAATCCATT
TCACATCAGATCTTTCAGATGGAAGTGAATGTGTTTTAGTCACAAAAGTGCCCCTGAAGCTCAGTTTACAC
AAGCAGAGAAAACAAACAGAGTAAGTTATGATGATGCTGATGAAGGTCTCCTCATGTCTCATGTCTCTCAC
ACAGATGCCAAACAACAGACAGAAGCGTGGCTTTAAGTGTAAGTTCTGCTGCGGCTGCTGCAGAGCTGGTG

TCTGTGGACTGTGCTGCAAGTTCTGAGGATTCCTGCTCCGGACAA
//
NRC215 (SEQ ID NO: 149)
AAGATGAAGACAATCAGTGTTGCAGTCACAGTGGCCGTCGTCCTCGTCTTTATTTGTATCCAGCAGAGCTC
TGCCTCCTTTCCTGAGGTAAGCACCTGACTTCAGATCGTTTAATTTGCTTGTTATCCATGAATCTCTCATC
AACATACTGAGACTTGATTCCTTCTTTATCAGGCACAAGAGCTGGAGGAGGCAGTGAGCAATGACAATGCA
GCTGCTGAGCATCAGGAGACACCAGTG
GACTCAGGGATGGTAGGTTCAGTTCACTGAATGGATCAATCCATTTCACATCAGATCTTTCAGATTGAAGT
GAATGTGTTTTAGTCACAAAAGTGCCCTGAAGCTCAGTTTACACAAGCAGAGAAAACAAACAGAGTAAGTT
ATGATGATGCTGATGAAGGTCTCCTCATGTCTCATGTCTCTCACACAGATTCCATACAACAGACAGAAGCG
TAGCTTTAAGTGTAAGTTCTGCTGCGGCTGCTGCAGAGCTGGTGTCTGTGGACTGTGCTGCAAATTCTGAG
GACCTGCCAGCA
//
NRC216 (SEQ ID NO: 150)
AAGATGAAGACATTCAGTGGTGCAGTCACAGTGGCCGTCGTGCTCGTCTTTATTTGTATCCAGCAGAGCTC
TGCCTCCTTTCCTGAGGTAAGCACCTGACTTCAGATCGTTTCATTTGCTTGTTATCCATGAATCTCTCATC
ATCATACTGAGACTTGATTCCTTCTTTATCAGGTACAAGAGCTGGAGGAGGCAGTGAGCAATGACAATGCA
GCTGCTGAACATCAGGAGACACCAGTTGACTCGTGGATGGTAGGTTCAGTTCACTGAATGGATCAATCCAT
TTCACATCAGATCTTTCAGATGGAAGTGAATGTGTTTTAGTCACAAAAGTGCCCTGAAGCTCAGTTTACAC
AAGCAGAGAAAACAAACAGAGTAAGTTATGATGATGCTGATGAAGGTCTCCTCATGTCTCATGTCTCTCAC
ACAGATGCCAAACAACAGACAGAAGCGTGGCTTTAAGTGTAAGTTCTGCTGCGGCTGCTGCAGAGCTGGTG
TCTGTGGACTGTGCTGCAAATTCTGAGGACCTGCCAGCA
//
NRC217 (SEQ ID NO: 151)
AAGATGAAGACATCAGTGGTTGCAGTCACAGTGGCCGTCGTGCTCGTCTTTATTTGTATCCAGCAGAGCTC
TGCCACCTTTCCTGAGGTAAGCACCTGACTTCAGATAGCTTCATTTGCTTGTTATCCATGAATCTCTCATC
AACATACTGAGACTTTATTCCTTCTTTATCAGGTACAAGAGCTGGAGGAGGCAGTGAGCAATGACAATGCA
GCTGCTGCGCATCAGGAGACATCAGTGGACTCGTGGATGGTAGGTTCAGTTCACTCAATGGATCAAACCAA
TTCACATCAGATCTTTCAGATGGAAGCGAATGTGTTTTAGTCAAAAAAGTGACCTGATGCTCAGTTTACAC
AAGCAGAGAAAACAAGCAGAGTAAGTTATGATGATGCTGATGAACGTGTCCTCATGTCTCATGTCTCTCAC
ACAGATGCCATACAACAGACCGAAGCGTAGCTTTAAGTGTAAGTTCTGCTGCGGCTGCTGCAGAGCTGGTG
TCTGTGGACTGTGCTGCAAATTCTGAGGATTCCTGCTCCAACAAC
//
NRC218 (SEQ ID NO: 152)
AAGATGAAGACATTCAGTGTGGCAGTCACAGTGGCCGTCGTGCTCGTCTTTATTTGTATCCAGCAGAGCTC
TGCCACCTTTCCTGAGGTAAGCACCTGACTTCAGATAGCTTCATTTGCTTGTTATCCATGAATCTCTCATC
AACATACTGAGACTTGATTTCTTCTTTATCAGGTACAAGAGCTGGAGGAGGCAGTGAGCAATGACAATGCA
GCCGCTGAACATCAGGAGACATCAGTGGACTCGTGGATGGTAGGTTCAGTTCACTCAATGGATCAAACCAA
TTCACATCAGATCTTTCAGATGGAAGTGAATGTGTTTTAGTCACAGAAGTGCCCTGATGCTCAGTTTACAC
AAGCAGAGAAAACAAGCAGAGTAAGTTATGATGATGCTGATGAACGTGTCCTCATGTCTCATGTCTCTCAC
ACAGATGCCATACAACAGACCGAAGCGTAGCTTTAAGTGTAAGTTCTGCTGCGGCTGCTGTAGAGCTGGTG
TCTGTGGACTGTGCTGCAAATTCTGAGGATTCCTGCTCCAACAAC
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NRC219 (SEQ ID NO: 153)
AAGATGAAGACATTCGTGGTTGCAGTCACAGTGGCCGTCGTGCTCGTCTTTATTTGTATCCAGCAGAGCTC
TGCCACCTTTCCTGAGGTAAGCACCTGACTTCAGATAGCTTCATTTGCTTGTTATCCATGAATCTCTCATC
AACATACTGAGACTTGATTCCTTCTTTATCAGGTACAAGAGCTGGAGGAGGCAGTGAGCAATGACAATGCA
GCCGCTGAACATCAGGAGACATCAGTGGACTCGTGGATGGTAGGTTCAGTTCACTCAATGGATCAAACCAA
TTCACATCAGATCTTTCAGATGGAAGTGACTGTGTTTTAGTCACAAAAGTGCCCTGATGCTCAGTTTACAC
AAGCAGAGAAAACAAGCAGAGTAAGTTATGATGATGCTGATGAACGTCTCCTCATGTCTCATGTCTCTCAC
ACAGATGCCATACAACAGACAGAAGCGTAGCTTTAAGTGTAAGTTCTGCTGCGGCTGCTGCAGAGCTGGTG
TCTGTGGACTGTGCTGCAAATTCTGAGGATTCCTGCTCCAACAAC
//
NRC220 (SEQ ID NO: 154)
AAGATGAAGACATCAGTGGTTGCAGTCACAGTGGCCGTCGTGCTCGTCTTTATTTGTATCCAGCAGAGCTC
TGCCACCTTTCCTGAGGTAAGCACCTGACTTCAGATAGCTTCATTTGCTTGTTATCCATGAATCTCTCATC
AACATACTGAGACTTTATTCCTTCTTTATCAGGTACAAGAGCTGGAGGAGGCAGTGAGCAATGACAATGCA
GCTGCTGCACATCAGGAGACATCAGTGGACTCGTGGATGGTAGGTTCAGTTCACTCAATGGATCAAACCAA
TTCACATCAGATCTTTCAGATGAAGTGACTGTGTTTTAGTCACAAAAGTGCCCTGATGCTCAGTTTACACA
AGCAGAGAAAACAAGCAGAGTAAGTTATGATGATGCTGATGAACGTGTCCTCATGTCTCATGTCTCTCACA
CAGATGCCATACAACAGACATAAGCGTAGCTTTAAGTGTAAGTTCTGCTGCGGCTGCTGCAGAGCTGGTGT
CTGTGGACTGTGCTGCAAATTCTGAGGATTCCTGCT
//

NRC221 (SEQ ID NO: 155)
AAGATAAGACATTCAGTGTTGCAGTCACAGTGGCCGTCGTGCTCGTCTTTATTTGTATCCAGCAGAGCTCT
GCCACCTTTCCTGAGGTAAGCACCTGACTTCAGATCGTTTCATTTGCTTGTTAGCCTTGAATCTCTCATCA
ACATACTGAGACTTGATTTCTTCTTTATCAGGTACAAGAGCTGGAGGAGGCAGTGAGCAATGACAATGCAG
CTGCTGAACATCAGGAGACATCAGTGGACTTGTGGATGGTAGGTTCAGTTCACTGAATGGATCAAACCAAT
TCACATCAGATCTTTCAGATGGAAGTGAATGTGTTTTAGTCACAAAAGTGCCCTGAAGCTCAGTTTACACG
AGCAGAGAAAACCAACACAGTAAGTTATGATGATGCTGATGAACGTCTCCTCATGTCTCATGTCTCTCACA
CAGATGCCATACAACAGACAGAAGCGTGGCTTTAAGTGTAAGTTCTGCTGCGGCTGCTGCAGCCCTGGTGT
CTGTGGACTTTGCTGCAGATTCTGAGGATTCCTGCTCCAACAAC
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NRC222 (SEQ ID NO: 156)
AAGATGAAGACATTCAGTGTTGCAGTCGCAGTGGCCGTCGTGCTCATCTTTATTTGTATCCAGCAGAGCTC
TGCCACCTTTCCTGAGGTAAGCACCTGACTTCAGATAGTTTCATTTGCTTGTTATCCATGAATCTCTCATC
AACATACTGAGACTTTATTCCTTCTTTATCAGGTACAAGAGCTGGAGGAGGCAGTGAGCAATGACAATGCA
GCTGCTGAACATCAGGAGACATCATTGGACTCATGGATGGTAGGTTCAGTTCACTCAATGGATCAAACCAA
TTCACATCAGATCTTTCAGATGGAAGTGACTGTGTTTTAGTCACAAAAGTGCCCTGATGCTCAGTTTACAC
AAGCAGAGAAAACAAGCAGAGTAAGTTATGATGATGCTGATGAACGTGTCCTCATGTCTCATGTCTCTCAC
ACAGATGCCATACAACAGACAGAAGCGTGGCTTTAAGTGTAAGTTCTGCTGCGGCTGCTGCAGAGCTGGTG
TCTGTGGACTGTGCTGCAAATTCTGAGGACCTGCCAGCA
//
NRC223 (SEQ ID NO: 157)
AAGATGAAGACATTCAGTGTTGCAGTCACAGTGGCCGTCGTGCTCGTCTTTATTTGTATCCAGCAGAGCTC
TGCCACCTTTCCTGAGGTAAGCACCTGACTTCAGATAGTTTCATTTGCTTGTTATCCATGAATCTCTCATC
AACATACTGAGACTTTATTCCTTCTTTATCAGGTACAAGAGCTGGAGGAGGCAGTGAGCAATGACAATGCA
GCTGCTGAACATCAGGAGACATCATTGGACTCATGGATGGTAGGTTCAGTTCACTCAATGGATCAAACCAA
TTCACATCAGATCTTTCAGATGGAAGTGAATGTGTTTTAGTCACAAAAGTGCCCTGATGCTCAGTTTACAC
AAGCAGAGAAAACAAGCAGAGTAAGTTATGATGATGCTGATGAACGTGTCCTCATGTCTCATGTCTCTCAC
ACAGATGCCATACAACAGACATAAGCGTAGCTTTAAGTGTAAGTTCTGCTGCGGCTGCTGCAGAGCTGGTG
TCTGTGGACTGTGCTGCAAATTCTGAGGACCTGCCAGCA
//
NRC224 (SEQ ID NO: 158)
AGATGAAGACATTCAGTGTTGCAGTCACAGTGGCCGTCGTGCTCGTCTTTATTTGTATCCAGCAGAGCTCT
GCCACCTTTCCTGAGGTAAGCACCTGACTTCAGATAGTTTCATTTGCTTGTTATCCATGAATCTCTCATCA
ACATACTGAGACTTGATTTCTTCTTTATCAGGTACAAGAGCTGGGGGAGGCAGTGAGCAATGACAATGCAG
CCGCTGAACATCAGGAGACATCAGTGGACTCGTGGATGGTAGGTTCAGTTCACTCAATGGATCAAACCAAT
TCACATCAGATCTTTCAGATGGAAGTGAATGTGTTTTAGTCACAAAAGTGCCCTGATGCTCAGTTTACACA
AGCAGAGAAAACAAGCAGAGTAAGTTATGATGATGCTGATGAACGTGTCCTCATGTCTCATGTCTCTCACA
CAGATGCCATACAACAGACCGAAGCGTAGCTTTAAGTGTAAGTTCTGCTGCGGCTGCTGCAGAGCTGGTGT
CTGTGGACTGTGCTGCAAATTCTGAGGACCTGCCAGCA
//
NRC225 (SEQ ID NO: 159)
AAGATGAAGACATTCAGTGTTGCAGTCACAGTGGCCGTCGTGCTCATCTTTATTTGTATCCAGCAGAGCTC
TGCCACCTCTCCTGAGGTACAAGGGCTGGAGGAGGCAGTGAGCAATGACAATGCAGCTGCTGAACATCAGG
AGACATCAGTGGACTCGTGGATGATGCCATACAACAGACAGAAGCGTGGCTTTAAGTGTAAGTTCTGCTGC
GGCTGCTGCAGGCCTGGTGTCTGTGGACTTTGCTGCAGATCCTGAGGATTCCTGCTCCAACAAC
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NRC226 (SEQ ID NO: 160)
AAGATGAAGACATTCAGTGTTGCAGTCACAGTGGCCGTCGTGCTCGTCTTTATTTGTATCCAGCAGAGCTC
TGCCACCTTTCCTGAGGTAAGCACCTGACTTCAGATCGTTTCATTTGCTTGTTAGCCTTGAATCTCTCATC
AACATACTGAGACTTGATTTCTTCTTTATCAGGTACAAGAGCTGGAGGAGGCAGTGAGCAATGACAATGCA
GCTGCTGAACATCAGGAGACATCAGTGGACTTGTGGATGGTAGGTTCAGTTCACTGAATGGATCAAACCAA
TTCACATCAGATCTTTCAGATGGAAGTGAATGTGTTTTAGTCACAAAAGTGCCCTGAAGCTCAGTTTACAC
GAGCAGAGAAAACCAACACAGTAAGTTATGATGATGCTGATGAACGTCTCCTCATGTCTCATGTCTCTCAC
ACAGATGCCATACAACAGACAGAAGCGTGGCTTTAAGTGTAAGTTCTGCTGCGGCTGCTGCAGACCTGGTG
TCTGTGGACTTTGCTGCAGATTCTGAGGATTCCTGCTCCAACAAC
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NRC227 (SEQ ID NO: 161)
AAGATGAAGACATTCAGTGTTGCAGTCACAGTGGCCGTCGTGCTCGTCTTTATTTGTATCCAGCAGAGCTC
TGCCACCTTTCCTGAGGTAAGCACCTGACTTCAGATCGTTTCATTTGCTTGTTAGCCTTGAATCTCTCATC
AACATACTGAGACTTGATTTCTTCTTTATCAGGTACAAGAGCTGGAGGAGGCAGTGAGCAATGACAATGCA
GCTGCTGAACATCAGGAGACATCAGTGGACTTGTGGATGGTAGGTTCAGTTCACTGAATGGATCAAACCAA
TTCACATCAGATCTTTCAGATGGAAGTGAATGTGTTTTAGTCACAAAAGTGCCCTGAAGCTCAGTTTACAC
GAGCAGAGAAAACCAACACAGTAAGTTATGATGATGCTGATGAACGTCTCCTCATGTCTCATGTCTCTCAC
ACAGATGCCATACAACAGACAGAAGCGTGGCTTTAAGTGTAAGTTCTGCTGCGGCTGCTGCAGTCCTGGTG

TCTGTGGACTTTGCTGCAGATTCTGAGGATTCCTGCTCCAAC
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NRC228 (SEQ ID NO: 162)
AAGATGAAGACATTCAGTGTTGCAGTCACAGTGGCCGTCGTGCTCGTCTTTATTTGTATCCAGCAGAGCTC
TGCCACCTTTCCTGAGGTAAGCACCTGACTTCAGATCGTTTCATTTGCTTGTTAGCCTTGAATCTCTCATC
AACATACTGAGACTTGATTTCTTCTTTATCAGGTACAAGAGCTGGAGGAGGCAGTGAGCAATGACAATGCA
GCTGCTGAACATCAGGAGACATCAGTGGACTCGTGGATGGTAGGTTCAGTTCACTGAATGGATCAAACCAA
TTCACATCAGATCCTTCAGATGGAAGTGAATGTGTTTTAGTCACAAAAGTGCCCTGAAGCTCAGTTTACAC
GAGCAGAGAAAACAAACACAGTAAGTTATGATGATGCTGATGAACGTCTCCTCATGTCTCATGTCTCTCAC
ACAGATGCCATACAACAGACAGAAGCGTGGCTTTAAGTGTAAGTTCTGCTGCGGCTGCTGCAGTCCTGGTG
TCTGTGGACTTTGCTGCAAATTCTGAGGACCTGCCAGCA
//
NRC229 (SEQ ID NO: 163)
AAGATGAAGACATTCAGTGTTGCAGTCACAGTGGCCGTCGTGCTCGTCTTTATTTGTATCCAGCAGAGCTC
TGCCACCTTTCCTGAGGTAAGCACCTGACTTCAGATCGTTTCATTTGCTTGTTAGCCTTGAATCTCTCATC
AACATACTGAGACTTGATTTCTTCTTTATCAGGTACAAGAGCTGGAGGAGGCAGTGAGCAATGACAATGCA
GCTGCTGAACATCAGGAGACATCAGTGGACTCGTGGATGGTAGGTTCAGTTCACTGAATGGATCAAACCAA
TTCACATCAGATCTTTCAGATGGAAGTGAATGTGTTTTAGTCACAAAAGTGCCCTGAAGCTCAGTTTACAC
GAGCAGAGAAAACAAACACAGTAAGTTATGATGATGCTGATGAACGTCTCCTCATGTCTCATGTCTCTCAC
ACAGATGCCATACAACAGACAGAAGCGTGGCTTTAAGTGTAAGTTCTGCTGCGGCTGCTGCAGACCTGGTG
TCTGTGGACTTTGCTGCAAATTCTGAGGACCTGCCAGCA
//
NRC230 (SEQ ID NO: 164)
AAGATGAAGACATTCAGTGTTGCAGTCACAGTGGCCGTCGTGCTCGTCTTTATTTGTATCCAGCAGAGCTC
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AGACATCAGTGGACTCGTGGATGATGCCATACAACAGACAGAAGCGTGGCTTTAAGTGTAAGTTCTGCTGC
GGCTGCTGCAGACCTGGTGTCTGTGGACTTTGCTGCAAATTCTGAGGACCTGCCAGCA
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NRC231 (SEQ ID NO: 165)
AAGATGAAGACATTCAGTGTTGCAGTCACAGTGGCCGTCGTGCTCGTCTTTATTTGTATCCAGCAGAGCTC
TGCCACCTTTCCTGAGGTACAAGAGCTGGAGGAGGCAGTGAGCAATGACAATGCAGCTGCTGAACATCAGG
AGACATCAGTGGACTCGTGGATGATGCCATACAACAGACAGAAGCGTGGCTTTAAGTGTAAGTTCTGCTGC
GGCTGCTGCAGGCCTGGTGTCTGTGGACTTTGCTGCAGATTCTGAGGATTCCTGCTCCAACAAC
//
NRC232 (SEQ ID NO: 166)
AAGATGAAGACATTCAGTGTTGCAGTCACAGTGGCCGTCGTGCTCGTCTTCATTTGTATCCAGCAGAGCTC
TGCCACCTTTCCTGAGGTAAGCACCTGACTTCAGATCGTTTCATTTGCTTGTTAGCCTTGAATCTCTCATC
AACATACTGAGACTTGATTTCTTCTTTATCAGGTACAAGAGCTGGAGGAGGCAGTGAGCAGTGACAATGCA
GCTGCTGAACATCAGGAGACATCAGTGGACTCGTGGATGGTAGGTTCAGTTCACTGAATGTGTTTTAGTCA
CAAAAGTGCCCTGAAGCTCAGTTTACACAAGCAGAGAAAACAAACAGAGTAAGTTATGATGATGCTGATGA
ACGTCTCCTCATGTCTCATGTCTCTCACACAGATGCCATACAACAGACAGAAGCGTAGCTTTAAGTGCAAG
TTCTGCTGCGGCTGCTGCAGACGTGGTGTCTGTGGACTGTGCTGCAAATTCTGAGGATTCCTGCTCCAACA
AC
//
NRC233 (SEQ ID NO: 167)
AAGATGAAGACTATCAGTGTTGCAGTCACAGTGGCCGTCGTGCTCCTCTTCATTTGTACCCAGCAGAGCTC
TGCCACCTTTCCTGAGGTAAGCACCTGACTTCAGATCGTTTCATTTGCTTGTTAGCCTTGAATCTCTCATC
AACATACTGAGACTTGATTTCTTCTTTATCAGGTACAAGAGCTGGAGGAGGCAGTGAGCAGTGACAATGCG
GCTGCTGAACATCAGGAGACATCAGTGGACTCGTGGATGGTAGGTTCAGTTCACTGAATGGATCAAACCAA
TTCACATCAGATCTTTCAGATGGAAGTGAATGTGTTTTAGTCACAAAAGTGCCCTGAAGCTCAGTTTACAC
AAGCAGAGAAAACAAACACAGTAAGTTATGATGATGCTGATGAACGTCTCCTCATGTCTCATGTCTCATGT
CTCTCACACAGATGCCATACAACAGACAGAAGCGTGGCTTTAAGTGCAAGTTCTGCTGCGGCTGCCGCTGT
GGTGCTCTCTGTGGACTGTGCTGCAAATTCTGAGGATTCCTGCTCCAACAAC
//
NRC234 (SEQ ID NO: 168)
AAGATGAAGACATTCAGTGTTGCAGTCACAGTGGCCGTCGTGCTCGTCTTCATTTGTATCCAGCAGAGCTC
TGCCACCTTTCCTGAGGTAAGCACCTGACTTCAGATCGTTTCATTTGCTTGTTAGCCTTGAATCTCTCATC
AACGTACTGAGACTTGATTTCTTCTTTATCAGGTACAAGAGCTGGAGGAGCCAGTGAGCAGTGACAATGCA
GCTGCTGAACATCAGGAGACATCGGTGGACTCGTGGATGGTAGGTTCAGTTCACTGAATGGATCAAACCAA
TTCACATCAGATCTTTCAGATGGAAGTGAATGTGTTTTAGTCACAAAAGTGCCCTGAAGCTCAGTTTACAC
AAGCAGAGAAAACAAACACAGTAAGTTATGATGATGCTGATGAACGTCTCCTCATGTCTCATGTCTCATGT
CTCTCACACAGATGCCATACAACAGACAGAAGCGTGGCTTTAAGTGCAAGTTCTGCTGCGGCTGCCGCTGT
GGTGCTCTCTGTGGACTGTGCTGCAAATTCTGAGGACCTGCCAGCA
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NRC235 (SEQ ID NO: 169)
AAGATGAAGACATTCAGTGTTGCAGTCACAGTGGCCGTCGTGCTCGTCTTTATTTGTTCCAGCAGAGCTCT
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ACATACTGAGACTTGATTTCTTCTTTATCAGGTACAAGAGCTGGAGGAGGCAGTGAGCAGTGACAATGCAG
CTGCTGAACATCAGGAGACATCAGTGGACTCGTGGATGGTAGGTTCAGTTCCCTGAATGGATCAAACCAAT
TCACATCAGATCTTTCAGATGGAAGTGAATGTGTTTTAGTCACAAAAGTGCCCTGAAGCTCAGTTTACACA
AGCAGAGAAAACAAACACAGTAAGTTATGATGATGCTGATGAACATCTCCTCATGTCTCATGTCTCATGTC
TCTCACACAGATGCCATACAACAGACAGAAGCGTGGCTTTAAGTGCAAGTTCTGCTGCGGCTGCCGCTGTG
GTGCTCTCTGTGGACTGTGCTGCAAATTCTGAGGACCTGCCAGCA
//
NRC236 (SEQ ID NO: 170)
ACGAGCTGACAGGAGCTGACAGGAGTCACCAGCAGAGTCAAAGAACTAAACAACTTAACTCAGTCAAACTC
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CTCTGCCACCTTTCCTGAGGTACAAGAGCTGGAGGAGGCAGTGAGCAATGACAATGCAGCTGCTGAGCATC
AGGAGACACCAGTGGACTCAGGGATGATGCCAAACAACAGACAGAAGCGCAGCGCCGATTGTTGGCCATGT
TGCAATCAAAATGGCTGTGGAACTTGCTGCAAGGTCTAAACAGACTCTTGGGCAGATCAATCCAGGTTCGT
CTTTCGTTGTCTCTCCGTGGAGTCGAACCAGAGACCTTCTCAGCCCATAGTCCAAGTTTCTGCCACTAGAC
CACCGCCTCTCCCTCATCAAATACTCAATGTTTTTCATTTTGTCTTAAAGTTCATTGAACTATAAACATAT
TTCTGGTAGAGCATGTGATAGTTTAATGGTGTTACTCATTGGTTCATGGTATAGTCAGATGTTCAGAGATG
TGATTATATCATCCACATATTTTCTCTGTTAAGGTGTACTGTCAATAAATGTCAATGCTCCTTTGAAAAAA
AAAAAAAAAAAAAAAC
//
NRC237 (SEQ ID NO: 171)
CGTGCTCGTCTTTATTTGTATCCAGCAGAGCTCTGCCACCTTTCCTGAGGTGAGCTCCTGACTTCAGATCG
TTTCATTTAGCTTGTTATCCATGAATCTCTCATCAACATACTGAGACTTGAATCCTTCTTTATCAGGTACA
GGAGCTGGAGGAGGCAGTGAGCAATGACAATGCAGCTGCTGAACATCAGGAGACATCAGTGGACTCATGGA
TGGTATGTTCAGTTCACTGAATGGATCAAACCAATTCACATCAGATCTTTCAGATGGAAGTGAATTTGTTT
TAGTCCCAAAAGTGCCCTGAAGCTCAGTTTACACAAGCAGAGAAAAACAAAACACAGTAAGTTATGATGAT
GCTGATGAACGTCTCCTCATGTCTCATGTCTCTCACACAGATGCCATACAACAGACAGAAGCGCAGCGCCG
AGTGTAGCTTCTGCTGCAATGAATCTGGCTGTGGAATTTGCTGCAAATTCTGAGGATTCCTGCTCCAACAA
CAAGGGCGAATTC
//
NRC238 (SEQ ID NO: 172)
AAGATGAAGACATTCAGTGTTGCAGTCACAGTGGCCGTCGTGCTCGTCTTTATTTGTATCCAGCAGAGCTC
TGCCACCTTTCCTGAGGTGAGCTCCTGACTTCAGATCGTTTCATTTAGCTTGTTATCCATGAATCTCTCAT
CAACATACTGAGACTTGAATCCTTCTTTATCAGGTACAGGAGCTGGAGGAGGCAGTGAGCAATGACAATGC
AGCTGCTGAACATCAGGAGACATCAGTGGACTCATGGATGGTATGTTCAGTTCACTGAATGGATCAAACCA
ATTCACATCAGATCTTTCAGATGGAAGTGAATTTGTTTTAGTCCCAAAAGTGCCCTGAAGCTCAGTTTACA
CAAGCAGAGAAAAACAAAACACAGTAAGTTATGATGATGCTGATGAACGTCTCCTCATGTCTCATGTCTCT
CACACAGATGCCATACAACAGACAGAAGCGCAGCGCCGAGTGTAGCTTCTGCTGCAATGAATCTGGCTGTG
GAATTTGCTGCAAATTCTGAGGACCTGCCAGCA
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NRC239 (SEQ ID NO: 173)
GTGGAGGAGCCAGTGAGCAGTGAGAATGGAGCAAATGAACACACATAAGATCTTTCGGATGGAAGTGTATG
TGTTTTAGTCACATGAGTGGCTCGAAGCTCAGTACACACGAGCAGAGAGAACGAACACAGTGTGTTTTATT
CTGCTTGTGTAAACTGAGCTTCAGTTTACACAAGCAGAGAAAACAAACACAGTAAGTTATGATGATGCTGA
TGAACGTCTCCTCATGTCTCATATCTCTCACACAGATGCCAAACAACAGACAGAAGCGTGGCTCTAATTGC
AAACCATGCTGCAATCATAATGGCTGTGGAACGTGCTGCGAAGTCTGAGGATTCCTGCTCCACA
//